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Validation Report 42

Determination of pesticide residues in wheat, rice, rye, and oat 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 sougth validated for 29 pesticides and metabolites by both gas and liquid chromatography combined with triple quadrupole in four different cereal matrixes (wheat, rice, rye and oat). The pesticides and/or metabolites included in the validation study are shown in Appendix 3.

2. Principle of analysis

Sample preparation

Blank samples of wheat, rice, rye, and oat were milled with a sieve at 1 mm and stored at -80°C. Five gram was weighted accurately in a 50 mL polypropylene PP tube. Ceramic homogenizers were inserted in each tube before adding 10 mL of cold water and 10 mL of acetonitrile. Samples were mechanically shaken for 5 minutes by a Ginogrinder. Prepared mixture of salts, containing 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H citrate sesquihydrate, were added to the samples. Tubes were shaken mechanically for another minute and then centrifuged for 10 minutes at 4500 rpm. Eight millilitre of supernatant were transferred in a clean tube and placed in -80°C freezer for at least 1 hour. After freezing-out the samples were removed from freezer, thawed and centrifuged at 5°C for 10 minutes at 4500 rpm.

Appropriate amount of extract was transfer for the LC analyses and another 6 mL extract were transferred to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$, shaken 30 seconds and centrifuged five minutes at 4500 rpm. After centrifugation step 4 ml was transfer in a clean 15 ml tubes containing 40 µl of 5% formic acid and analysed on GC.

GC-MS/MS parameters

For gas chromatographic separation, a Thermo Scientific™ Trace™ 1310 Gas Chromatograph coupled to a Thermo Scientific™ TriPlus™ RSH autosampler was used. The samples were injected in a programmable temperature vaporizer (PTV) mode through a PTV baffle liner 2×2.75×120 mm for Thermo GCs (Siltek). The injection volume was 1 µL and the injection temperature was set to 70°C. Helium as used as carrier gas at a flow of 1.2 $ml.min^{-1}$. The analytes were separated on a TG-5SILMS (capillary column of 30 m long, 0.25 mm inner diameter and a film thickness of 0.25 µm). The oven temperature program was as follows: 60°C for 1.5 min, up to 90°C at 25°C/min for 1.5 min, up to 180°C at 25°C /min, then up to 280°C at 5 °C/min and finally up to 300°C at 10°C/min and

for 12 min. The total runtime was 42 min. For the mass spectrometric analysis, a Thermo Scientific™ TSQ™ 8000 Evo was used. The instrument has been upgraded with and Advanced Electron ionisation source, (AEI). The AEI source was operated with an electron energy of 50 eV. The analyses were performed by a triple quadrupole operating in the SRM mode (Selected Reaction Monitoring). The source temperature was set at 300°C, and the transfer line, at 280°C.

LC-MS/MS parameters

For liquid separation, a LC system Thermo Ultimate 3000 and the mass spectrometer Bruker EVOQ. The analytes were separated on a Accuity UPLC BEH C18 1.7 µm, 2.1*100 mm reversed-phase column. The injection volume was **2** µl. The eluents consisted of milli-q water with 0,1% formic acid and 5 mM ammonia solution (A eluent) and methanol (B eluent) and a flow rate of 0.4 ml/min was applied. The analytes were separated using a gradient elution program. In this program the column is equilibrated with 2% B eluent before injection. At the time of injection the B eluent is increased to 35% within 0.1 min and then increased further reaching 98% at a run time of 7 min. The 98% of B eluent is then maintained for 3 minutes before the proportion is lowered again to 2% within 0.1 min and maintained until a total run time of 13 min in order to prepare the column for the next injection. The mass spectrometer was operated in multiple reaction monitoring mode and using both + and negative electrospray ionization.

3. Validation

Validation design

The method was validated for 28 compounds (pesticides or/and metabolites) in four different matrices (wheat, rice, rye, and oat). The validation was performed on 5-6 replicates at each of the four cereals matrices, and at four spiking levels of 0.002, 0.005, 0.01 and 0.05 mg/kg. Extraction of a blank sample were included for all commodities.

Calibration curves and linearity

Linearity study was performed by using matrix-matched calibration curve prepared in 5 concentrations for each one of the compounds within the range of 0.33 to 100 µg/L. The calibration curves were fitted to linear function and the deviation of the back–calculated concentration of the calibration standards from the true concentrations were within ±20%.

All quantifications were performed using bracketing matrix matched calibration curves.

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 was based on the lower calibration levels for the low spike samples.

Accuracy – Recovery

Recovery values were calculated as average recovery of 5-6 replicates for each level (0.002, 0.005, 0.01, and 0.05 mg/kg) and matrices. Accepted recovery range was between 70 and 120% (following SANTE document)[3]. Values outside this range have been accepted if the precision data was satisfactory.

Precision – repeatability and internal reproducibility

Repeatability and internal reproducibility were calculated for all pesticides and degradation products on all four spiking levels (0.002, 0.005, 0.01 and 0.05 mg/kg) as given in ISO 5725-22. Accepted values were ≤ 20%.

Limit of quantification, LOQ

The Limit of quantification (LOQ) was determined as the lowest spiked level for which the acceptance criteria were met (average relative recovery between 70 and 120% and precision lower than or equal to 20%), and ion ratios for sample extracts were within ±30% (relative) of average of relevant calibration standards.

4. Results and conclusion

A total of 28 compounds were successfully validated using QuEChERS method. Seven compounds were validated on both GC-MS/MS and LC-MS/MS, 19 compounds were only validated on GC-MS/MS and 16 only on LC-MS/MS. All the validation data for the pesticides and/or metabolites and four different matrices are presented in appendix 2.

An LOQ of 0.002 mg/kg was achieved for 28 compounds. An LOQ of 0.005 was achieved for two compounds (Dichlorophen and XMC).

The majority of the combined uncertainties were lower than 50%.

5. 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 – Part 2. 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/ 11312 /2021.

Appendix 1A. GC-MS/MS conditions

Retention time, Rt, precursor mass, product mass, and collision energy (CE).

Pesticide	Rt	Precursor	Product	CE	Precursor	Product	CE
Aspon	14.6	253	115	15	294	145.9	20
Atrazine-Desethyl	11.0	145	110.1	10	187.1	58.1	10
Benzoylprop-ethyl	21.5	260	145	30	260	186	15
Butachlor	17.0	176.1	134.1	10	176.1	146.1	20
Butamifos	17.0	200	65.1	20	202	185	10
Butylate	9.0	174.1	146.1	5	217.1	156.2	5
Crimidine	9.6	142.1	106.1	10	144.1	106.1	10
Cyhalofop-butyl	23.6	256.1	120.1	10	357.2	120.1	20
Dicapthon	15.1	216	123	15	216	201	10
Dithiopyr	14.0	258.1	230.1	5	306.1	258.1	10
Fenoprop-methylester	11.6	286	200	10	223	159	10
Fluchloralin	12.0	264	160.1	10	264	206.1	5
Fluridone	27.6	328.1	189.1	45	328.1	259.1	25
Nitrothal-isopropyl	14.8	194	120	15	236.1	148	15
Pentanochlor	14.1	140.1	77.1	15	141.1	106.1	10
Plifenate	13.2	175	111	15	177	113	15
Profluralin	11.8	318.1	199.1	10	318.1	284.1	5
Tiocarbazil	14.8	156.2	41.1	15	156.2	57.1	10
Triflumizole metabolite (FM-6-1)	11.6	201	136	15	235.1	188	25
zeta- Cypermethrin	26.7	163	127	10	181	152	20
Aspon	14.6	253	115	15	294	145.9	20

Appendix 2B. LC-MS/MS conditions;

Ionisation mode, retention time, Rt, precursor mass, product mass, and collision energy (CE).

Pesticide	ESI mode	Rt	Precursor	Product	CE	Precursor	Product	CE
Atrazine-Desethyl	+	3.1	188	146	-14	188	79	134
Aziprotryne	+	5.5	226	68.2	-26	226	156	-11
Benzoximate	+	6.7	366	106	-24	366	319.9	-4
Butamifos	+	6.6	333	96	-27	333	180	-8
Crimidine	+	3.0	172	136	-16	172	95.2	-20
Cyhalofop-butyl	+	6.8	375	256	-17	375	120	-28
Dichlormid	+	4.0	208	41.4	-14	208	81.2	-8
Dichlorophen	-	5.0	268	127	18	268	66.2	16
Dithiopyr	+	7.0	402	354	-12	402	271.7	-28
Metamitron-desamino	+	2.7	188	160	-14	188	77.2	-29
Methabenzthiazuron	+	4.7	222	165	-14	222	150	-29
Naptalam	+	4.0	292	144	-8	292	149	-17
Nitrothal-isopropyl	+	1.0	313	230.8	-10			
Pentanochlor	+	5.0	240	142.1	-14	240	107	-27
Siduron	+	5.5	233	94.2	-17	233	137	-14
Triflumizole metabolite (FM-6-1)	+	3.3	295	73.2	-15	295	43.4	-19
XMC	+	4.3	180	123	-8	180	108	-28

Appendix 2. Validation results

Recoveries (Rec), repeatability (RSD_r), internal reproducibility (RSDR), expanded uncertainty (U) without correcting for recoveries and Limit of Quantification (LOQ) for pesticides validated on four cereal commodities, wheat (W) rice(Ri), rye (Ry) and oat(O), using QuEChERS.

	Pesticide	Spike level 0.002 mg/kg					Spike level 0.005 mg/kg					Spike level 0.01 mg/kg					Spike level 0.05 mg/kg					LOQ	Matrices
		Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %		
GC	**Aspon**	76	18	40	95	41	75	15	21	66	21	79	11	16	53	16	82	9	18	52	19	0.002	Ry,Ri,W,O
GC	**Atrazine-Desethyl**	89	6	20	47	20	72	6	30	82	30	77	7	14	54	14	81	7	10	44	11	0.002	Ry,Ri,W,O
LC	**Atrazine-Desethyl**	76	15	17	59	18	84	10	13	42	14	79	9	14	50	14	87	13	27	61	27	0.002	Ry,Ri,W
LC	**Aziprotryne**	89	8	25	55	25	92	15	20	44	20	89	14	14	36	14	98	8	10	21	10	0.002	Ry,Ri,W,O
LC	**Benzoximate**	86	19	19	47	19	96	13	14	30	14	93	12	13	29	13	97	8	9	20	9	0.002	Ry,Ri,W,O
GC	**Benzoylprop-ethyl**	88	6	19	47	20	90	16	19	43	19	71	8	29	82	29	85	6	8	35	8	0.002	Ry,Ri[2],W[1],O
GC	**Butachlor**	81	6	19	54	20	74	7	27	76	28	78	8	15	53	15	84	8	12	41	13	0.002	Ry,Ri,W,O
GC	**Butamifos**	84	12	13	42	14	78	4	22	63	22	81	6	10	42	10	85	7	13	39	13	0.002	Ry,Ri,W,O
LC	**Butamifos**	105	11	41	84	42	91	11	38	80	39	75	18	25	72	26	89	8	21	48	21	0.002	Ry[2],Ri,W,O
GC	**Butylate**	84	17	17	49	18	73	14	30	82	31	75	9	13	56	14	80	9	14	49	14	0.002	Ry,Ri,W,O
GC	**Crimidine**	83	10	16	48	17	79	7	15	52	16	77	9	18	58	18	80	9	15	50	15	0.002	Ry,Ri[2],W,O
LC	**Crimidine**	82	20	27	67	28	93	10	12	28	12	89	9	9	28	9	97	13	16	34	17	0.002	Ry,Ri,W
GC	**Cyhalofop-butyl**	82	6	7	39	8	77	4	26	70	26	78	8	13	52	13	82	6	8	40	8	0.002	Ry,Ri,W,O
LC	**Cyhalofop-butyl**	88	20	22	52	23	95	11	11	24	11	91	10	10	27	10	98	10	20	40	20	0.002	Ry,Ri,W
GC	**Dicapthon**	93	14	13	31	14	78	10	20	59	20	81	10	14	48	14	82	13	20	55	21	0.002	Ry,Ri,W,O
LC	**Dichlormid**	107	11	21	46	22	101	17	22	45	23	90	20	25	55	26	99	9	11	23	11	0.002	Ry,Ri[1],W[1],O
LC	**Dichlorophen[1]**	87	37	45	96	46	92	17	20	44	21	85	11	12	38	12	90	7	13	33	13	0.002	Ry,Ri,W
GC	**Dithiopyr**	88	5	11	34	12	75	5	36	89	37	81	7	18	53	18	87	6	8	32	9	0.002	Ry,Ri,W,O
LC	**Dithiopyr**	103	9	50	103	51	107	12	15	34	16	94	9	9	22	9	100	8	14	29	15	0.002	Ry,Ri,W,O[2]
GC	**Fenoprop-methylester**	91	7	14	34	14	74	9	29	79	29	74	9	19	66	20	79	6	8	45	8	0.002	Ry,Ri,W,O
GC	**Fluchloralin**	98	9	9	20	10	80	12	20	57	21	81	8	13	47	14	81	10	20	57	21	0.002	Ry,Ri[2],W,O
GC	**Fluridone**	82	13	17	51	18	85	5	14	41	14	79	7	16	54	17	83	7	10	40	10	0.002	Ry,Ri[2],W,O

	Pesticide	Spike level 0.002 mg/kg					Spike level 0.005 mg/kg					Spike level 0.01 mg/kg					Spike level 0.05 mg/kg					LOQ	Matrices
		Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %	Rec %	RSDr %	RSDR %	U %	Cu %		
LC	**Metamitron-desamino**	83	8	26	62	26	81	9	13	45	13	78	9	8	47	9	88	12	18	44	19	0.002	Ry,Ri,W
LC	**Methabenzthiazuron**	91	9	11	29	11	93	12	13	29	13	86	12	11	36	12	93	7	11	27	12	0.002	Ry,Ri,W,O
LC	**Naptalam**	99	15	34	71	35	96	12	16	33	16	93	13	13	31	13	98	9	10	21	10	0.002	Ry,Ri,W,O
GC	**Nitrothal-isopropyl**	85	13	16	45	17	81	9	18	53	18	75	9	19	64	19	79	8	13	50	14	0.002	Ry,Ri[2],W,O
LC	**Nitrothal-isopropyl**	115	11				103	21				88	20				102	4				0.002	O
GC	**Pentanochlor**	81	10	23	61	24	77	9	26	70	27	81	7	13	47	13	86	6	10	34	10	0.002	Ry,Ri,W,O
LC	**Pentanochlor**	91	9	20	45	21	96	8	12	26	13	91	12	11	29	11	96	8	11	24	11	0.002	Ry,Ri,W,O
GC	**Plifenate**	84	19	32	73	33	70	16	38	99	39	74	9	17	63	17	74	9	11	56	11	0.002	Ry,Ri,W,O
GC	**Profluralin**	92	11	12	30	12	72	10	29	81	29	78	8	14	53	14	75	8	13	58	14	0.002	Ry,Ri,W,O
LC	**Siduron**	83	14	27	66	28	90	7	16	38	16	95	9	12	26	12	95	9	11	24	11	0.002	Ry,Ri,W
GC	**Tiocarbazil**	67	13				86	8	19	48	19	74	9	20	66	20	78	5	10	48	10	0.002	Ry[1],Ri[2],W[2],O
GC	**Triflumizole metabolite (FM-6-1)**	84	15	26	62	27	76	11	33	83	34	78	14	19	60	20	85	7	9	35	9	0.002	Ry,Ri,W,O
LC	**Triflumizole metabolite (FM-6-1)+**	96	13	17	36	18	98	11	17	34	17	93	13	12	29	13	101	11	17	34	17	0.002	Ry,Ri,W,O
LC	**XMC[1]**	93	22	27	56	27	96	9	16	34	16	92	12	11	28	11	100	8	14	29	14	0.002	Ry,Ri,W,O
GC	**zeta- Cypermethrin**	73	9	17	65	18	76	5	10	52	10	77	8	13	54	13	78	7	12	51	13	0.002	Ry,W,O

[1] LOQ = 0.005 mg/kg

[2]LOQ = 0.01 mg/kg

Appendix 3: Flowchart of the QuEChERS method for cereal samples