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Validation Report 34

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[1] method combined with GC-MS/MS and LC-MS/MS. The method was validated for 14 pesticides and metabolites by both LC-MSMS and GC-MSMS in wheat, rye, oat and rice. The pesticides included in the validation study and the reason for including them is presented in Appendix[3].

2. Sample preparation and extraction method

The cereal samples are milled with a sieve at 1 mm. The extraction procedure is outlined in Appendix 4. Water and acetonitrile are added and the sample is shaken. Salt and buffer mixture is added and the sample is shaken again. After centrifugation the supernatant is transferred to a clean tube and put in -80 degree freezer for minimum 1 hour. 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 At this point an aliquot is withdrawn and filtered, diluted 1:1 with acetonitrile and analysed by LC-MS/MS.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. The final extracts are analysed by GC-MS/MS. Crude extract withdrawn before PSA clean-up was analysed by LC-MS/MS.

3. Instrumentation

3.1. GC-MS/MS

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 (99.999%) was used as carrier gas at a flow of 1.2 $ml.min^{-1}$. The analytes were separated on a DB5-MS 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 electron ionization (EI) source was used with an electron energy of 70 eV. The analyses were performed by a triple quadrupole operating in the multiple

reaction-monitoring mode (MRM). The source temperature was set at 300°C, and the transfer line, at 280°C.

3.2. LC-MS/MS

The pesticide residues analysis were also performed by LC-(ESI)MS/MS. The LC system employed was a Thermo Ultimate 3000 and the mass spectrometer was a Bruker EVOQ. The analytes were separated on a Accuity UPLC BEH C18 1.7 µm, 2.1*100 mm reversed-phase column. The injection volumne was 1 µl. The eluents consisted of milli-q water with 0,1% formic acid and 5 mM ammonia solution (A eluent) and methanol (B elutent) and a flow rate of 0.4 ml/min was applied. The analytes were separated using a gradient elution programme. The column is equlibrated with 2% B eluent before injection. At the time of injection the propotion of B elutent is increased to 35% within 0.1 min and then inceased 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 positive and negative electrospray ionisation.

4. Validation design

The method was sought validated for 14 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 four spiking levels; 0.002, 0.005, 0.01 and 0.05 mg/kg. A blank sample of each cereal commodity is included.

Linearity range

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. Thus, good linearity was observed within the relevant concentration range.

Recovery

The recovery was determined from recovery studies in which samples were spiked at four concentration levels (0.002, 0.005, 0.01 and 0.05 mg/kg) with the relevant pesticides. The average relative recovery for all pesticides were between 70 and 120%[3].

Precision-repeatability and reproducibility

Repeatability was calculated for all pesticides and degradation products on all four spiking levels (0.002, 0.005, 0.01 and 0.05 mg/kg). Repeatability (RSD_r) in this validation was calculated as given in ISO 5725-2[2] from the 5-6 replicate determinations. To evaluate the reproducibility, the intra-laboratory precision (RSD_R) between different cereal matrices (rye, wheat, oat, and rice). Both repeatability and reproducibility were less than 20% for all pesticides included in this study.

Uncertainty

The expanded uncertainty is calculated using the following formula:

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

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

The combined uncertainty calculated using the following formula:

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

Where RSD is the repeatability (RSD_r),
RSD^2/n is the uncertainty of the bias,
n is the number of replicates included in the bias

LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (recovery, repeatability, reproducibility, and uncertainty) were meet. 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 14 pesticides or metabolites using LC-MSMS and GC-MSMS are presented in appendix 2. The lowest LOQ achieved was 0.002 mg/kg for 8 compounds (oxathiapiprolin, phosmet-oxon, quinmerac, spinosad_A, spinosad_D, tioxazafen, tribenuron-methyl, and tridemorph). The LOQ achieved for three pesticides (nicotine, quinoclamine, and sintofen) was 0.005 mg/kg. The LOQ of topramezone was 0.01 mg/kg. The LOQ achieved for paraoxon-methyl and thiophanate-methyl is 0.05 mg/kg.

The combined uncertainties were lower than 50% for all compounds, indicating that recovery for correction is not needed.

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,

4 Working document on pesticides to be considered for inclusion in the national control programmes to ensure compliance with maximum residue levels of pesticides residues in and on food of plant and animal origin, European Commission, Brussels, 2017. SANCO 12745 2013 rev 11.

Appendix 1a. MRM transitions for compounds validated by GC-MS/MS

Name	RT	Parent Mass	Product Mass	Collision Energy
Nicotine	8.28	162.1	84.1	10
Nicotine	8.28	162.1	161.2	10
Tioxazafen	14.84	228	111	10
Tioxazafen	14.84	228	119.1	10

Appendix 1b. MRM transitions for compounds validated by LC-MS/MS

Compound Name	RT	ESI mode	Precursor ion	Product ion	Collision Energy
Oxathiapiprolin	5.53	Positive	543	500.1	21
Oxathiapiprolin	5.53	Positive	543	163	39
Paraoxon-methyl	3.31	Positive	265	202	35
Paraoxon-methyl	3.31	Positive	265	127	40
Phosmet-oxon	3.28	Positive	302	160	21
Phosmet-oxon	3.28	Positive	302	133	31
Quinmerac	2.86	Positive	222.3	204	12
Quinmerac	2.86	Positive	222.3	141.1	30
Quinoclamine	3.59	Positive	208.6	100.3	55
Quinoclamine	3.59	Positive	208.6	103.2	36
Sintofen	4.4	Positive	375.1	234	24
Sintofen	4.4	Positive	375.1	208	38
Spinosad_A	5.67	Positive	733	142	21.5
Spinosad_A	5.67	Positive	733	189	30
Spinosad_D	5.92	Positive	747	142	22
Spinosad_D	5.92	Positive	747	189	27.5
Thiophanate-methyl	3.73	Positive	342.78	151.12	20
Thiophanate-methyl	3.73	Positive	342.78	93.15	50
Tioxafen	6.6	Positive	229.4	111.1	12
Tioxafen	6.6	Positive	229.4	82.9	10
Topramezone	2.39	Positive	364.4	334	8
Topramezone	2.39	Positive	364.4	125	19
Tribenuron-methyl	4.55	Positive	396.08	154.97	17
Tribenuron-methyl	4.55	Positive	396.08	181	22
Tridemorph	5.38	Positive	298.5	130.2	22
Tridemorph	5.38	Positive	298.5	98.2	24

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
GC	Nicotine						29	7	15	146	15	27	8	20	151	21	25	4	13	153	14	0.005
LC	Oxathiapiprolin	103	14	18	38	19	104	16	20	41	20	106	17	20	42	20	113	17	18	46	19	0.002
LC	Paraoxon-methyl											*54*	*24*	*22*	*102*	*23*	76	19	66	147	69	0.05
LC	Phosmet-oxon	118	7	9	41	9	120	14	18	55	19	103	14	17	36	17	95	8	19	40	19	0.002
LC	Quinmerac	97	8	18	37	18	95	8	16	34	16	97	15	14	29	14	81	15	15	48	15	0.002
LC	Quinoclamine						95	13	20	42	21	87	19	20	49	21	103	14	20	42	21	0.005
LC	Sintofen						119	9	19	54	19	107	11	21	45	21	98	16	18	37	18	0.005
LC	Spinosad_A	120	14	19	56	20	118	15	24	61	25	114	15	22	53	22	118	13	25	63	25	0.002
LC	Spinosad_D	118	17	19	54	20	122	13	14	52	14	113	18	20	50	21	126	17	19	66	20	0.002
LC	Thiophanate-methyl	*122*	*36*	*55*	*120*	*56*	91	21	27	58	28	57	27	38	115	38	60	19	23	94	24	0.05
GC	Tioxazafen	76	5	6	51	6	76	4	6	50	6	82	3	5	37	6	90	3	7	25	7	0.002
LC	Tioxazafen	93	9	10	25	11	97	7	11	23	11	95	15	17	37	18	100	6	8	17	9	0.002
LC	Topramezone											62	8	20	88	21	51	26	25	111	26	0.01
LC	Tribenuron-methyl	98	12	13	28	14	101	10	14	29	14	102	16	19	40	20	96	16	19	40	20	0.002
LC	Tridemorph	95	10	37	77	38	86	8	35	80	37	94	14	22	48	23	113	8	19	49	20	0.002

Appendix 3. List of compounds included in the validation study including reason for inclusion.

Compound	**Reason for including in validation study 2019**
Nicotine	In working document
Oxathiapiprolin	In working document
Paraoxon-methyl	Parathion methyl is on EU MACP and paraoxon-methyl is included in the residue definition. lower LOQs to be tested.
Phosmet-oxon	Phosmet in EU MACP and phosmet-oxon is included in the residue definition. lower LOQs to be tested.
Quinmerac	Art. 12 evaluation request. Authorized in EU. MRL set at 0.1* mg/kg for cereals. To be tested whether lower LOQ can be set.
Quinoclamine	In the working document (snaco 12745 2013 rev 11, Annex III) because it is of interest for cumulative risk assessment.
Sintofen	Art. 12 evaluation request
Spinosad	In EU MACP. lower LOQs to be tested.
Thiophanate-methyl	In EU MACP. lower LOQs to be tested.
Tioxazafen	MRL application for corn, soybean and cotton seeds for imported commodities and EU evaluation initiated.
Topramezone	In working document in list of "Previously listed in Chapter 4.2.3 (Voluntary in Reg. (EU) N° 788/2012)".
Tribenuron-methyl	Authorized in EU and relevant for cereals. Lower LOQs to be tested.
Tridemorph	Found in screening analysis of apples. Not authorized in EU. Relevant for use on cereal crops.

Appendix 4: Chart 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.