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

Validation Report 37

Determination of pesticide residues in oat, rice, rye and wheat,
by LC-MS/MS and GC-MS/MS

(QuEChERS method)

Ederina Ninga
Mette Erecius Poulsen
Elena Hakme
January 2022

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

2. Principle of analysis

Sample preparation

Blank samples of oat, rice, ray and wheat were milled with a sieve at 1 mm and stored at -80°C. Five gram were 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 minute 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 cirate 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 an 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^{TM} 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 1 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 programme. In this program the column is equlibrated with 2% B eluent before injection. At the time of injection the 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 + and negative electrospray ionization.

3. Validation

Validation design

The method was validated for 35 compounds (pesticides or/and metabolites) in four different matrices (oat, rice, rye, and wheat). The validation was performed on 5 replicates of each of the four cereals matrices in four different spiking levels; 0.002, 0.005, 0.01 and 0.05 mg/kg. Extraction of a blank sample were included for all commodity.

Calibration curves and linearity

Linearity study were performed by using matrix-matched calibration curve prepared in 5 different concertation 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 calibration curves using matrix matched calibration curve.

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.

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 matrixes. Accepted values for recovery were recoveries in the range 70-120% (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 was calculated for all pesticides and degradation products on all three 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 determinate as the lowest spiked level for which the acceptance criteria were meet (average relative recovery in percentage between 70 and 120 and precision lower than or equal 20%), and ion ratios for sample extracts were within ±30% (relative) of average of relevant calibration standards.

4. Results and conclusion

The validation results obtained for the 44 compounds in four different cereal matrices (oat, rice, rye, and wheat). Only 35 compounds were validated. Nine compounds (1-Naphthylacetic acid, 8-Hydroxyquenoline, aminopiralid, cinerin I, cinerin II, clopyralid, jasmoline I, pyrethrin I, picloram) did not fulfil the criteria for validation using the above levels and method conditions.

Fourteen out of 35 compounds were validated by both GC and LC. All the data for the pesticides and/or metabolites and four different matrices are presented in appendix 2.

The lowest LOQ achieved were 0.002 mg/kg for 16 compounds in GC-MS/MS, only two compounds pebulate and prometon had the LOQ 0.005 mg/kg. However, these two compounds obtained an LOQ of 0.002 in LC-MS/MS. For 32 compound validated in LC-MS/MS, 24 compounds achieved an LOQ of 0.002 mg/kg. For chlorbromuron, crufomate, flumetsulam, warfarin the LOQ was 0005 mg/kg,

and for pinoxaden was 0.01 mg/kg. The LOQ achived for jasmoline II and pyrethrin II was 0.05 mg/kg.

Some compounds did not have the same sensitivity in all the matrices. DBCP (1,2-dibromo-3-chloropropane) had a LOQ of 0.002 mg/kg in rice and rye but for wheat and oat it was 0.005. Dioxacarb was validated in oat, rice and rye (LOQ was 0,002 mg/kg) but not in wheat. Imazapyr had an LOQ of 0.002 in oat, rye and wheat but in rice the LOQ was 0.005. Pinoxade was validated in rice and rye (LOQ was 0,01 mg/kg) but was not validated in oat and wheat.
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/12682/2019.

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

Compound name	RT	Precursor ion	Product ion	Collision Energy	Precursor ion	Product ion	Collision Energy
2-3-5-Trimethacarb	10.84	136.1	121.1	10	136.1	77.1	30
Bromacil	14.32	205	188	10	207	134	20
Chlorbufam	11.81	223.1	127.1	10	164.1	128.1	10
Crufomate	15.14	276.1	182.2	10	256.1	226.1	20
Diclofop-methyl	20.81	252.9	162.1	15	340	184.1	25
DBCP	6.31	156.9	75.1	10	156.9	39.1	20
Esprocarb	14.43	222.1	91.1	10	162.2	91.1	10
Fenobucarb	10.41	150.1	121.1	5	121	77	20
Fenothiocarb	16.83	160.1	72.1	10	160.1	55.1	15
Isofetamid	23.79	165	107	20	165	150	10
Lenacil	20.15	153	136.1	10	153	135.1	10
Mepronil	19.51	269.2	119.1	10	119.1	65.1	20
Oxadiazon	17.69	174.9	112	10	302	175	10
Pebulate	9.11	161.1	128.2	10	161.1	57.1	10
Prometon	11.64	210.2	168.1	10	210.2	112.1	10
Pyributicarb	21.39	165.1	108.1	10	181.1	108.1	10
Sebuthylazine	12.82	200.1	122.1	10	202.1	134.1	10

[1] 1,2-dibrom-3-chlorpropane

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

Compound name	ESI mode	RT	Precursor ion	Product ion	Collision Energy	Precursor ion	Product ion	Collision Energy
2-3-5-Trimethacarb	+	4.77	194	137.1	9	194	122.1	25
Boturon	+	4.69	237	84.2	12	237	126	26
Bromacil	+	3.88	261	204.9	11	261	187.8	26
Chlorbromuron	+	5.4	295	205.8	16	295	125	30
Crufomate	+	6.12	292.7	237	15	292.7	108.1	24
Desmetryn	+	3.72	214	82.2	27	214	57.3	27
Dimefuron	+	5.04	339.8	72.3	20	339.8	167	18
Dioxacarb	+	2.74	224.2	123.1	13	224.2	167.1	5
Esprocarb	+	7.17	266.4	91.2	18	266.4	71.3	13
Fenobucarb	+	5.23	208.3	95.2	11	208.3	152.1	4
Flumetsulam	+	2.52	326	129.1	20	326	109.1	41
Fluometuron	+	4.72	233	72	13	233	233	11
Imazapyr	+	2.54	262	217.1	16	262	69.3	23
Ioxynil	-	4.98	370	126.9	27	370	242.8	20
Isofetamid	+	5.84	359.5	125	24	359.5	210	6
Isoxaben	+	5.56	332.4	165	18	332.4	91.2	24
Jasmoline II	+	7.07	374.5	163.1	7	374.5	161.1	6
Lenacil	-	4.68	233	151	19			
Mepronil	+	5.54	270	119.1	20	270	91.2	37
Oxadiazon	+	7.25	346	304	8	346	219.9	16
Pebulate	+	6.7	204	57.3	14	204	128.2	8
Pinoxaden	+	6.62	401	317.1	19	401	57.3	20
Prometon	+	3.9	226.3	142.1	19	226.3	184.1	14
Pyraclonil	+	4.48	314.7	168.9	24	314.7	159	19
Pyrethrin II	+	6.81	373.2	161.1	7	373.2	133.1	16
Pyributicarb	+	7.3	331.4	181	10	331.4	108.1	24
Pyrimedifen	+	6.9	378.9	186	20	378.9	150	31
Rimsulfuron	+	4.19	431	182	18	431	325	12
Sebuthylazine	+	5.2	230	174	15	230	68.2	31
Tebuthiuron	+	4.03	229	172.1	14	229	116.1	23
Terbumeton	+	3.93	226	170.1	14	226	69.2	33
Warfarin	+	5.4	309	163	11	309	251	16

Appendix 3: Flowchart of the QuEChERS method for cereal samples