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Validation Report 39

Determination of pesticide residues in Black Solder Larvae by LC-MS/MS

(QuEChERS µSPE celan-up)

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CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method followed by µSPE clean-up combined with LC-MS/MS. Tweenty one pesticides frequently found in the found in in different food commodities in EU[1] were chosen for the purpose of this study. The pesticides included in the validation study are shown in Appendix 1.

2. Principle of analysis

Sample preparation

Blank samples of freeze dry black soldier larvae were homogenized using in a blender with dry ice. For the validation 2 gram test portion from the homogenise sample were weighted accurately in a 50 mL polypropylene PP tube. Ceramic homogenizers were inserted in each tube before adding 10 mL solution of 4/1 acetonitrile/water. Samples were mechanically shaken for 5 minute by a Ginogrinder at 1000 rpm followed by 3 minute centrifugation at 4500 rpm. The extracts were decanted into the 15 mL PP tubes containing containing 2 g MgSO4, 0.5g NaCl, 0.5 g Na3 citrate dihydrate and 0.25 g Na2H citrate sesquihydrate for salting out. The tubes were capped well, briefly shaken by hand, and then shaken on mechanically for another five minutes at 1000 rpm and then centrifuged for 3 minutes at 4500 rpm. Lastly, 1 mL of each extract was pipetted into a glass autosampler vials for automated µSPE clean-up appendix 3.

The vials with the uncleaned extract were placed Stand-Alone Thermo Scientific™ TriPlus™ RSH™ multi-purpose autosampler (based on a PAl3_RTC autosampler from CTC Analytics). Chromeleon 7 version software was used to program and operate the device.In the appendix 3 are given all the steps for the workflows used in the celan up step. After the preparation the same sample vial was injected in LS-MS/MS.

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 was equlibrated with 2% B eluent before injection. At the time of injection, the B elutent was 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 was 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 21 compounds in black soldier fly at four different spiking levels.The validation was performed on 6 replicates of each spiking levels; 0.005, 0.01, 0.05 and 0.1 mg/kg. Blank samples were prepared in parallel with with the spied samples during the validation and were used for the preparation of the calibration curve.

Calibration curves and linearity

Linearity studies 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 is 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 (0.005, 0.01, 0.05, and 0.1 mg/kg) and matrixes. Accepted values for recovery were recoveries in the range 70-120% (following SANTE/11312/2021)[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 three spiking levels (0.005, 0.01, 0.05 and 0.1 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

Validation results obtained for the 21 pesticides or metabolites using LC-MS/MS are presented in the table 1. All compounds were validated, nineteen with and LOQ at 0.005 kg/kg, thiodiocarb at 0.01 mg/kg and finally iprodione was validated at 0.1 mg/kg.

5. References

1 EFSA (European Food Safety Authority), 2022. The 2020 European Union report on pesticide residues in food. EFSA Journal, 20(3): 7215, 58 pp.

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. LC-MS/MS conditions

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

Compound	Rt	ESI	Precursor	Product	CE	Precursor	Product	CE
Acephate	1.8	+	184	143	-12			
Acetamiprid	2.7	+	223	56	-10	223	90	-29
Boscalid	5.4	+	343	271	-24	343	307	-13
Buprofezin	7.0	+	306	116	-14	306	201	-8
Chlorpyrifos	7.4	+	350	198	-16	352	200	-18
Clofentezine	6.6	+	303	102	-30	303	138	-12
Diazinon	6.4	+	305	97	-30	305	153	-20
Epoxiconazole	5.9	+	330	101	-30	330	121	-18
Etofenprox	8.3	+	394	135	-23	394	177	-14
Fipronil	6.2	-	435	330	13	435	250	42
Fluopyram	5.8	+	397	173	30	397	208	22
Hexaconazole	6.6	+	314	70	-13	314	160	-25
Imidacloprid	2.5	+	256	175	-17	256	209	-14
Iprodione	6.1	+	330	101	-25	330	245	-12
Malathion	5.5	+	331	99	-18	331	127	-10
Phosmet	5.0	+	335	133	-36	335	160	-17
Propamocarb	1.8	+	189	74	-23	189	102	-13
Pymetrozine	1.7	+	218	105	-15	218	79	-20
Tebuconazole	6.3	+	308	70	-13	308	125	-32
Thiodicarb	4.2	+	355	88	-9	355	108	-12
Triazophos	5.7	+	314	119	-30	314	162	-17
Trifloxystrobin	6.8	+	409	145	-36	409	186	-11

Appendix 2: Validation results

Recoveries (Rec), repeatability (RSD_r) and Limit of Quantification (LOQ) for pesticides validated on Black Solder Larvea (BSL) using QuEChERS-µSPE.

Compound	0.005 mg/kg		0.01 mg/kg		0.05 mg/kg		0.1 mg/kg		LOQ
	Rec %	RSDr %	Rec %	RSDr %	Rec %	RSDr %	Rec %	RSDr %	
Acephate	66	10	65	5	69	10	65	6	0.005
Acetamiprid	72	6	77	13	84	13	82	9	0.005
Boscalid	57	12	72	8	81	7	76	7	0.005
Buprofezin	68	7	72	3	76	4	71	4	0.005
Chlorpyrifos	73	9	76	5	76	4	71	4	0.005
Clofentezine	51	6	58	5	69	5	65	5	0.005
Diazinon	70	13	72	6	75	3	72	5	0.005
Epoxiconazole	70	8	69	8	71	6	69	3	0.005
Etofenprox	62	11	71	5	75	7	71	4	0.005
Fipronil	71	13	72	11	77	8	74	4	0.005
Fluopyram	77	11	74	3	74	6	71	3	0.005
Hexaconazole	59	17	59	15	70	9	68	7	0.005
Imidacloprid	81	8	79	10	80	11	78	5	0.005
Iprodione	*103*	*67*	*67*	*27*	*78*	*33*	67	18	*0.1*
Malathion	74	3	72	4	78	5	75	6	0.005
Phosmet	70	13	76	9	76	13	77	8	0.005
Propamocarb	63	8	43	9	41	11	31	14	0.005
Pymetrozine	34	10	37	9	39	3	40	4	0.005
Tebuconazole	59	13	61	13	75	11	68	6	0.005
Thiodicarb	-	-	69	13	67	13	64	12	0.01
Triazophos	76	5	75	3	76	4	73	3	0.005
Trifloxystrobin	75	7	75	3	77	3	75	7	0.005

[1] **Results in italic do not fulfil the validation requirement and are not validated at this level.**

Appendix 3: Flowchart of the QuEChERS-µSPE clean-up method for BSL larvae.