Validation of new MRM pesticides included in the Working Document SANCO/12745/2013 by QuEChERS extraction method

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1. Aim and scope

This document describes the validation data for seven new pesticides included in the Working document SANCO/12745/2013 using QuEChERS extraction method by LC-MS/MS in tomato, orange and avocado.

2. Short description

Validation was evaluated in terms of accuracy (recovery) and precision (repeatability). Linearity and matriz effects were also studied. Homogenous samples were extracted using QuEChERS extraction method, which was adapted in the case of avocado. The obtained extracts were then analyzed by LC-MS/MS.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes of 10 µL to 5000 µL and 1 mL to 5 mL
- 50 ml and 15 ml PTFE centrifuge tubes
- Vortex
- Shaker
- Centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 3300 rpm
- Concentration workstation
- Injection vials, 2 ml, suitable for LC and GC auto-sampler

4. Chemicals

- Acetonitrile ultra-gradient.
- Trisodium citrate dihydrate
- Disodium hydrogenocitratesesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Primary secondary amine (PSA)
- Supel QuE Z-Sep
- C18
- Ammonium formate
- Ultra-pure water
- Methanol HPLC grade

- Formic acid
- Pesticides analytical standards

5. Procedure

5.1. Sample preparation

Following Document No. SANTE/2019/12682, the sample was homogenised by cryogenic milling at its arrival to the laboratory.

5.2. Recovery experiments for method validation

Individual pesticide stock solutions (1000–2000 mg/L) were prepared in acetonitrile and were stored in screw-capped glass vials in the dark at -20 °C.

For spiking, the representative portions of previously homogenised sample were weighed in teflon tubes, where they were spiked homogenously with the appropriate amount of the working standard solution in acetonitrile.

The validation method was performed at two fortification levels (0.01 and 0.10 mg/kg). Five replicates were analysed at each level.

5.3. Extraction procedure

QuEChERS

1. Weigh 10 g ± 0.1 g of sample in 50 mL PTFE centrifuge tube.
2. Add 10 mL of acetonitrile and 10 µL of 10 mg/L carbendazim-d3, malathion-d10 and TPP (procedure internal standards).
3. Shake the sample using an automatic axial shaker for 4 min.
4. Add 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate and 0.5 g of disodium hydrogenocitrate sesquihydrate.
5. Shake the samples again in the automatic shaker for 4 min.
6. Centrifuge the tubes at 3700 rpm for 5 min.
7. Transfer 5 mL of the supernatant to a 15 mL PTFE tube containing:
    a. 750 mg magnesium sulphate and 125 mg PSA for matrices with high water content.

b.750 mg magnesium sulphate and 125 mg Z-Sep for matrices with high fat content.

8. Vortex the tube for 30 sec.

9. Centrifuge the tubes at 3700 rpm for 5 min.

10. Add 40 µL of formic acid 5% in acetonitrile to option a in step 7.

11. Analysis: dilute 100 mL extract with 400 mL of water containing dimethoate-d6 at 0.050 mg/L (Injection Internal Standard). This way, 1 mL of sample extract represents 0.2 g of sample.

5.4. Measurement

LC system was operated in multiple reaction monitoring mode (MRM). Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of the target molecules. For confirmation of the studied compounds, two SRM transitions and a correct ratio between the abundances of the two optimized SRM transitions (SRM2/SRM1) were used, along with retention time matching. The mass transitions used are presented in **Appendix I**.

5.5. Instrumentation and analytical conditions for the LC- MS/MS system

5.5.1. 1290 UHPLC (Agilent)

- Column: Zorbax Eclipse Plus C8 2.1x100 mm and 1.8 µm particle size (Agilent)
- Mobile phase A: Water (0.1% formic acid, 5mM ammonium formate, 2% MeOH)
- Mobile phase B: Methanol (0.1% formic acid, 5mM ammonium formate, 2% H_2O)
- Column temperature: 35°C
- Flow rate: 0.3 mL/min
- Injection volume: 5 µL.

Mobile phase gradient for pesticides analysed

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	100	0
2	80	20
15	0	100
18	0	100

Re-equilibration with initial phase: 2.5 minutes

5.5.2. 6490 triple quadrupole system (Agilent)

- Ionisation mode: Positive mode and negative mode
- Capillary (positive and negative): 3000 V
- Nebulizer: 45 psi
- Nozzle: 400 V
- Drying gas flow: 13 L/min
- Drying gas temperature: 120°C
- Sheath gas flow: 10 L/min
- Sheath gas temperature: 375°C
- High Pressure RF (positive): 150 V
- High Pressure RF (negative): 110 V
- Low Pressure RF (positive): 60 V
- Low Pressure RF (negative): 60 V

6. Validation of the method

6.1. Recoveries and within-laboratory reproducibility

The results corresponding to the mean recovery (n=5) and within-laboratory reproducibility in terms of relative standard deviation (RSDr) at two fortification levels (0.01 and 0.10 mg/kg) are summarized in **Appendix II** (**Table 1**). All recovery results are within the range 70-120% (RSD≤20%).

6.2. Linearity

Linearity of the LC-MS/MS system was evaluated by assessing the signal responses of the target analytes from matrix-matched calibration solutions prepared by spiking blank extracts at six concentration levels, from 0.005 to 0.500 mg/L. In all cases, coefficient of determination (R^2) was higher than 0.99. Linearity ranges for all pesticides are summarized in **Appendix II** (**Table 2**).

6.3. Matrix effects

Matrix effects were assessed by comparison of the slopes of six-point matrix-matched calibration curves with the slopes of the calibration curves in solvent (LC). For values (in absolute terms) between 0 and 20 %, matrix effect was considered low; a moderate matrix effect would have values between 20 % and 50 %, and for compounds with a value above 50 %, matrix effect was considered strong. Values of matrix effects are summarized in **Appendix II** (**Table 2**). It´s important to recall that extracts were diluted five times prior to analysis; therefore, matrix-matched calibration curves contained 0.2 g of sample per mL. Only dinotefuran and oxathiapiprolin showed a remarkable matrix effect. Dinotefuran presented a strong signal suppression in orange and moderate in avocado. Oxathiapiprolin presented a strong signal suppression in avocado.

7. References

- Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Document Nº SANTE/2019/12682.
- http://www.eurl-pesticides.eu
- 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. SANCO/12745/2013

APPENDIX I: MASS TRANSITIONS

Detection and chromatographic parameters for the selected compounds analysed by LC-MS/MS.

No.	Name	t_R (min)	Cone voltage (V)	Precursor (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)	CE 1 (eV)	CE 2 (eV)	Polarity
1	Dinotefuran	3.28	380	203.1	129.1	114.1	9	9	Positive
2	Fenobucarb	10.882	380	208.2	151.9	95.1	5	20	Positive
3	Fenpicoxamid	13.348	380	615.3	515	238.9	13	25	Positive
4	Oxathiapiprolin	11.217	380	540.2	522	500	29	29	Positive
5	Quinalphos	12.122	380	299.1	270.8	242.8	10	10	Positive
6	Tolfenpyrad	13.534	380	384.1	197	170.9	25	20	Positive
7	Triallate	13.935	380	306.01	145	86	25	15	Positive

APPENDIX II: VALIDATION RESULTS.

Table 1. Accuracy data (as % recovery) and precision data (as repeatibility RSDr, n=5) at 0.005 and 0.050 mg/ kg for tomato, orange, avocado by using QuEChERS citrate.

No.	Compund	Tomato				Orange				Avocado			
		0.01 mg/kg		0.10 mg/kg		0.01 mg/kg		0.10 mg/kg		0.01 mg/kg		0.10 mg/kg	
		Recov (%)	RSD (%)	Recov (%)	RSD (%)	Recov (%)	RSD (%)	Recov (%)	RSD (%)	Recov (%)	RSD (%)	Recov (%)	RSD (%)
1	Dinotefuran	100	1	100	3	70	12	97	6	105	4	105	2
2	Fenobucarb	102	2	97	2	86	1	99	1	96	11	96	10
3	Fenpicoxamid	117	6	101	9	105	6	102	8	94	15	108	17
4	Oxathiapiprolin	100	3	103	6	107	3	97	3	97	12	104	8
5	Quinalphos	99	2	99	2	86	2	98	2	103	11	97	9
6	Tolfenpyrad	102	3	100	1	90	3	106	2	101	14	98	10
7	Triallate	102	7	95	2	86	9	103	5	103	13	92	15

Table 2. Linearity range, coefficient of determination and matrix effects for selected matrices studied by using QuEChERS citrate. Negative values of matrix effects mean suppression of the signal, and positives values, enhancement.

No.	Compund	Linear Range (mg/ kg)				R^2			Matrix effects (%)		
		Solvent	Tomato	Orange	Avocado	Tomato	Orange	Avocado	Tomato	Orange	Avocado
1	Dinotefuran	0.005-0.1	0.005-0.1	0.005-0.1	0.005-0.1	0.9999	0.9999	0.9995	-6	-78	-38
2	Fenobucarb	0.005-0.5	0.005-0.5	0.005-0.5	0.005-0.5	0.9999	0.9998	0.9997	-3	-6	2
3	Fenpicoxamid	0.005-0.5	0.005-0.3	0.005-0.1	0.005-0.5	0.9993	0.9932	0.9998	12	8	-5
4	Oxathiapiprolin	0.005-0.1	0.005-0.1	0.005-0.1	0.005-0.1	0.9990	0.9981	0.9991	-13	-16	-48
5	Quinalphos	0.005-0.5	0.005-0.5	0.005-0.5	0.005-0.5	0.9993	0.9996	0.9980	0	-1	3
6	Tolfenpyrad	0.005-0.5	0.005-0.5	0.005-0.5	0.005-0.5	0.9995	0.9997	0.9990	2	-10	2
7	Triallate	0.005-0.5	0.005-0.5	0.005-0.5	0.005-0.5	0.9998	0.9988	0.9999	6	-7	4