Validation data of five selected pesticides using QuEChERS by liquid chromatography tandem mass spectrometry

CONTENTS

1. Aim and scope

This report describes a validation data of five pesticides using a multiresidue method (QuEChERS) by LC-MS/MS in tomato, pepper and orange.

2. Short description

The analysis of pesticide residues was performed by using QuEChERS method.

The homogeneous sample is extracted with acetonitrile. After salts addition the mixture is shaken intensively and centrifuged for phase separation. An aliquot of the organic phase is taken for the clean-up. Extracts are shaken by vortex and a small aliquot is diluted with milliQ water.

Pesticide list is shown in the next table.

Table 1. Pesticide List.

Compound	Status in Annex I (Reg. EC 1107/2009)	Category
Cadusafos	Out	Insecticide, Nematcide
Fenpyrazamine	In	Fungicide
Fluopyram	In	Fungicide
Isoprothiolane	Out	Fungicide
Spinoteram	Pending	Insecticide

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes of 30 µL to 500 µL and 1 mL to 3 mL.
- 50 ml PTFE centrifuge tubes with screw caps
- 15 ml PTFE centrifuge tubes with screw caps
- Vortex
- Automatic axial extractor
- Centrifuge, suitable for the centrifuge tubes employed in the procedure and capable of achieving at least 3700 rpm
- Syringes, e.g. 2 mL disposable syringes
- Syringe filters, 0.45 µm pore size
- Injection vials, 2 ml, suitable for LC auto-sampler
- Volumetric flasks

4. Chemicals

- Acetonitrile ultra-gradient grade
- Formic acid
- Trisodium citrate dihydrate
- Sodium chloride
- Disodium hydrogencitrate sesquihydrate
- Anhydrous magnesium sulphate
- Ultra-pure water
- Pesticides standards
- Primary secondary amine bonded silica (PSA), bulk material
- C_{18}

5. Procedure

5.1. Sample preparation

Following Document No. SANCO/12495/2011, the sample was perfectly homogenised by grinding finely at its arrival to the laboratory.
Sample was frozen for its storage immediately after grinding it.

5.2. Recovery experiments for method validation

The samples employed in validation studies did not contain any of the pesticides analysed.

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

For spiking, 40 g representative portions of previously homogenised sample were weighed and transferred to a crystalliser, where they were fortified homogenously with the adequate volume of the working standard solution in acetonitrile.

The validation method was performed at two fortification levels (0.010 mg/Kg and 0.100 mg/Kg). Five replicates were analysed at each level.

5.3. Extraction

1. Weigh 10 g ± 0.1 g of sample in 50 mL PTFE centrifuge tube.
2. Add 10 mL of acetonitrile and 50 µL of a mix of triphenyl phosphate (TPP) and malathion-d_{10} (surrogate standards) at 10 mg/L.
3. Shake in automatic axial extractor for 4 minutes.
4. Add 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodiumcitrate dehydrate and 0.5 g of disodium hydrogencitrate sesquihydrate.
5. Shake in automatic axial extractor for 4 minutes.
6. Centrifuge for 5 min at 3700 rpm.
7. Transfer 3 mL of supernatant into 15 mL PTFE centrifuge tube containing 750 mg magnesium sulphate, 125 mg of PSA and 125 mg of C_{18} and shake in a vortex 30 s.
8. Centrifuge for 5 min at 3700 rpm.
9. Add to the extract 30 µL of 5% formic acid in acetonitrile.
10. Filter the sample thorough 0.45 µm PTFE filter and add a volume of dimethoate-d_6 (Injection standard) to obtain in the vial of 0.010 mg/kg.
11. Dilute an aliquot of the filtered extract 5 times for LC analysis with milliQ water.

With this treatment, 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 optimised 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. HPLC Agilent 1200

- Column: Agilent Zorbax SB, C8, 4.6 mm x 150 mm, 5 µm
- Mobile phase A: 0.1% formic acid in acetonitrile
- Mobile phase B: 0.1% formic acid in ultra-pure water
- Flow rate: 0.6 mL/min
- Injection volume: 10 µL

Mobile phase gradient used:

Time [min]	Mobile phase A	Mobile phase B
0	20%	80%
3	20%	80%
30	100%	0%
33	100%	0%

Re-equilibration with initial mobile phase: 7 minutes.

5.5.2. QqQ MS/MS Agilent 6490

- Ionisation mode: positive
- ESI source gas temperature: 120 °C
- Gas flow: 15 L/min
- Nebuliser gas: nitrogen
- Nebuliser gas pressure: 35 psi
- Sheath gas temperature: 375 °C
- Sheath gas flow: 12 L/min
- Capillary voltage: 3500 V
- Collision gas: nitrogen
- Nozzle voltage: 300V

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 (RSD) at both fortification levels are summarized in Appendix II.

Document N° SANCO/12495/2011 recommends mean recovery values within the range 70-120% and RSD<20%. All the validation results fulfil the criteria for the acceptance mentioned above.

6.2. Limits of quantitation

Document N° SANCO/12495/2011 defines limit of quantitation as the lowest validated spike level meeting the method performance acceptability criteria. LOQs are summarized in Appendix II (Table 2).

6.3. Linearity

Linearity of the LC-(QqQ)MS system was evaluated by assessing the signal responses of the target analytes from matrix-matched calibration solutions prepared by spiking blank extracts at five concentration levels; 0.010, 0.020, 0.050, .0100 and 0.200 mg/kg.

The criterion for the acceptance of the linearity is a correlation coefficient (r^2) equal or higher than 0.95. In all cases, coefficient of determination (r^2) was higher than 0.99. Linearity ranges for all pesticides in all matrices are summarized in Appendix II (Table 3).

This report aims to provide information to laboratories which will analyse these pesticides residues in fruit and vegetables or are interested in it.

7. References

- **European Committee for Standardization/Technical Committee 275 (Standards under development) (2007) Foods of plant origin: Determination of pesticide residues using GC–MS and/or LC–MS(/MS) following acetonitrile extraction/partitioning and cleanup by dispersive SPE–QuEChERS method.** European Committee for Standardization, Brussels.
- **Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed** (Document N° SANCO/12495/2011)

- **http://www.eurl-pesticides.eu**

Appendix I: Mass transitions

Mass transitions and ionisation mode used for the compounds analysed by LC-MS/MS.

Compound	SRM1	SRM2	Ionization mode
Cadusafos	271.0 / 158.7	271.0 / 130.9	ESI (+)
Fenpyrazamine	332.1 / 272.0	332.1 / 230.2	ESI (+)
Fluopyram	397.0 / 208.1	397.0 / 172.9	ESI (+)
Isoprotiolane	291.0 / 230.7	291.0 / 188.9	ESI (+)
Spinetoram	748.0 / 98.0	748.0 / 142.0	ESI (+)

Appendix II: Validation results

Table 2. Recoveries % (RSD) at 0.010 and 0.100 mg/kg (n=5) in the three matrices.

Compound	Tomato		Pepper		Orange	
	0.010 mg/kg	0.100 mg/kg	0.010 mg/kg	0.100 mg/kg	0.010 mg/kg	0.100 mg/kg
Cadusafos	120 (5)	96 (9)	105 (9)	108 (2)	83 (12)	77 (5)
Fenpyrazamine	115 (9)	90 (9)	97 (9)	104 (1)	99 (11)	97 (1)
Fluopyram	120 (3)	97 (9)	99 (10)	108 (3)	109 (11)	111 (1)
Isoprotiolane	112 (3)	98 (9)	103 (10)	110 (1)	105 (9)	106 (1)
Spinetoram	100 (4)	92 (3)	71 (7)	75 (7)	72 (3)	78 (20)

Table 3. Limits of quantification, linearity range and correlation coefficient for the selected matrices studied.

	Tomato			Pepper			Orange		
Compound	LOQ (mg/kg)	Linear range (mg/kg)	r^2	LOQ (mg/kg)	Linear range (mg/kg)	r^2	LOQ (mg/kg)	Linear range (mg/kg)	r^2
Cadusafos	0.010	0.010 - 0.200	0.9985	0.010	0.010 - 0.200	0.9991	0.010	0.010 - 0.200	0.9977
Fenpyrazamine	0.010	0.010 - 0.200	0.9984	0.010	0.010 - 0.200	0.9993	0.010	0.010 - 0.200	0.9980
Fluopyram	0.010	0.010 - 0.200	0.9993	0.010	0.010 - 0.200	0.9988	0.010	0.010 - 0.200	0.9977
Isoprotiolane	0.010	0.010 - 0.200	0.9986	0.010	0.010 - 0.200	0.9996	0.010	0.010 - 0.200	0.9981
Spinetoram	0.010	0.010 - 0.200	1.0000	0.010	0.010 - 0.200	0.9982	0.010	0.010 - 0.200	0.9996