

Validation of a multiresidue method based on QuEChERS extraction for the analysis of chlorpyrifos in banana by LC- and GC- MS/MS

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1. Aim and scope

This document reports the validation data in banana for chlorpyrifos, a broad-spectrum organophosphorus insecticide and acaricide used to control soil and foliage pests, using a QuEChERS-based multiresidue method by GC-MS/MS and LC-MS/MS.

2. Short description

Homogenous samples were extracted the citrate QuEChERS method. The obtained extracts were then analyzed by GC-MS/MS and LC-MS/MS.

Validation of the method was performed in terms of accuracy (recoveries at 0.002 mg/kg and 0.005 mg/kg), repeatability (five replicates), matrix effect and linearity.

3. Apparatus and consumables

- Automatic pipettes, suitable for handling volumes from 1 μ L to 5 mL
- Graduated 10 mL pipette
- 50 mL and 15 mL PTFE centrifuge tubes
- Vortex Shaker IKATM 4 Basic
- Axial shaker Agytax SR1 CP57
- Centrifuge Orto Alresa Consul 21, suitable for the centrifuge tubes employed in the procedure and capable of achieving 4000 rpm
- Concentration workstation
- Injection vials, 2 mL, suitable for LC and GC auto-sampler
- Amber vials, 4 mL

4. Chemicals

- Acetonitrile ultra-gradient grade
- Trisodium citrate dihydrate
- Disodium hydrogenocitrate sesquihydrate
- Sodium chloride
- Anhydrous magnesium sulphate
- Primary secondary amine (PSA)
- Ammonium formate
- Ultra-pure water

- Methanol HPLC grade
- Formic acid
- Ethyl acetate
- Pesticide analytical standards

5. Procedure

5.1. Recovery experiments for method validation

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

For spiking, representative portions of the previously homogenised banana sample were spiked homogeneously with the appropriate amount of the working standard solution in acetonitrile. The recovery experiments were performed at two spiking levels (0.002 and 0.005 mg/kg). Five replicates were analysed at each level.

5.2. Sample extraction (QuEChERS)

1. Weigh 10 g of homogenate sample (after cryogenic milling) in a 50-mL PTFE centrifuge tube.
2. Add 10 mL acetonitrile and 10 µL of a mixture of a 10 mg/L mixture of deuterated surrogate standards (carbendazim-D₃, dichlorvos-D₆ and malathion-D₁₀).
3. Shake the samples in an Agitax axial extractor for 4 min.
4. Add 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate.
5. Shake the samples in an Agitax axial extractor for 4 min.
6. Centrifuge at 4000 rpm for 5 min.
7. Transfer a 5-mL aliquot of the supernatant to a 15 mL PTFE tube containing 750 mg anhydrous magnesium sulphate and 125 mg PSA.
8. Vortex the tubes for 30 sec.
9. Centrifuge at 4000 rpm for 5 min.
10. Acidify with 10 µL formic acid 5 % per mL of extract.

5.3. Vial preparation

Extraction method	GC-MS/MS	LC-MS/MS
QuEChERS	Solvent change to ethyl acetate	5-fold dilution with ultrapure water

During vial preparation, dimethoate-D₆ (LC) or lindane-D₆ (GC) were added as internal standards (50 µg/L).

5.4. Methodology

Both LC and GC systems were 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 (Table 1 for LC-MS/MS and Table 2 for GC-MS/MS parameters).

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, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 35 °C
- Flow rate: 0.3 mL/min
- Injection volume: 5 µL

Mobile phase gradient for pesticides analysis:

Time [min]	Mobile phase A	Mobile phase B
0	100 %	0 %
2	80 %	20 %
15	0 %	100 %
18	0 %	100 %

Re-equilibration time with initial mobile phase set for 2.5 minutes.

5.5.2. 6490A 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

5.6. Instrumentation and analytical conditions for the GC- MS/MS system

5.6.1. Intuvo 9000 GC system (Agilent)

- Column: 2 Planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- Injection mode: splitless
- Ultra-inert inlet liner with a glass wool frit from Agilent
- Injection volume: 1 µl
- Injector temperature: 80 °C hold for 0.1 min, then up to 300 °C at 600 °C/min, hold for 5 min and then to 250 °C at 100 °C/min.
- Carrier gas: Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2.
- Carrier gas purity: 99.999 %
- Oven temperature: 60 °C for 0.5 min, up to 170 °C at 80 °C/min, and up to 310 °C at 20 °C/min.

5.6.2. 7010 triple quadrupole (Agilent)

- Ionisation mode: electron impact ionisation
- Temperature of the transfer line: 280 °C

- Temperature of ion source: 280 °C
- Collision gas: nitrogen
- Collision gas purity: 99.999 %
- Solvent delay: 2.6 minutes

6. Results

The validation results for chlorpyrifos in banana matrix are summarised below. The recovery and repeatability studies were conducted using five replicates at two spiking levels (0.002 and 0.005 mg/kg). The linearity was evaluated by assessing the signal response of chlorpyrifos from matrix-matched calibration solutions prepared by spiking blank banana extracts at six concentration levels from 0.002 to 0.200 mg/L. In both cases, the coefficient of correlation (R^2) was higher than 0.99.

Method	Mean rec. 0.002 mg/kg (RSD) (%)	Mean rec. 0.005 mg/kg (RSD) (%)	Linear range (mg/kg)
GC-MS/MS	95 (5)	98 (7)	0.002-0.200
LC-MS/MS	107 (9)	105 (7)	0.002-0.200

In **Figure 1** the chromatographs at 0.002 mg/kg of chlorpyrifos in banana by gas chromatography coupled to tandem mass spectrometry are represented

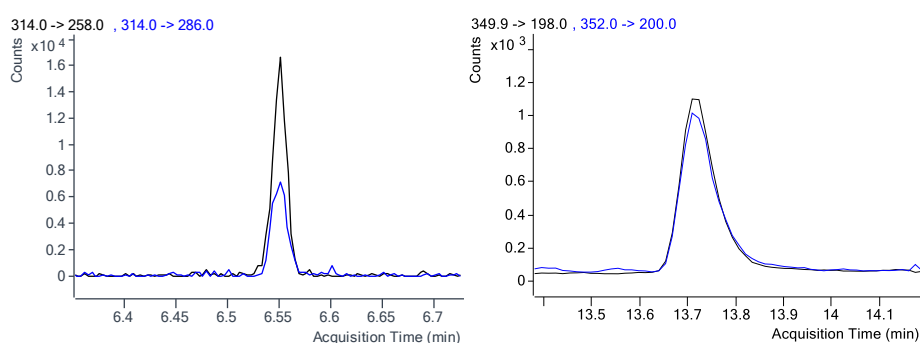


Figure 1. Chlorpyrifos chromatographic peaks by GC-MS/MS (left) and by LC-MS/MS (right) at 0.002 mg/kg.

7. References

- SANCO/ 12682/2019. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.
Available at: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf
- EURL-FV multiresidue method using QuEChERS followed by GCQqQ/MS/MS and LC-QqQ/MS/MS for fruits and vegetables.
https://www.eurl-pesticides.eu/library/docs/fv/CRLFV_Multiresidue_methods.pdf

APPENDIX: MASS TRANSITIONS

Table A1. Detection and chromatographic parameters for the LC-MS/MS instrument.

Name	t_R (min)	Cone voltage (V)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)	Polarity
Chlorpyrifos	13.79	380	352.0	200.0	20	349.9	198	20	Positive
Carbendazim-D ₃	4.16	380	195.1	159.8	20	195.1	131.9	20	Positive
Dichlorvos-D ₆	8.51	380	226.9	132.9	20	226.9	115.0	20	Positive
Dimethoate-D ₆	5.99	380	236.0	205.0	4	236.0	131.0	16	Positive
Malathion-D ₁₀	11.32	380	341.1	132.0	12	341.1	100.0	24	Positive

t_R : retention time

CE: collision energy

Table A2. Detection and chromatographic parameters for the GC-MS/MS instrument.

Name	t_R (min)	Precursor ion 1 (m/z)	Product ion 1 (m/z)	CE 1 (eV)	Precursor ion 2 (m/z)	Product ion 2 (m/z)	CE 2 (eV)
Chlorpyrifos	6.62	314.0	286.0	5	286.0	271.0	16
Dichlorvos-D ₆	3.43	191.0	115.0	20	191.0	99.0	15
Lindane-D ₆	5.61	224.0	187.0	5	224.0	150.0	20
Malathion-D ₁₀	6.45	183.0	151.0	3	183.0	132.0	5

t_R : retention time

CE: collision energy