Validation Data for the Analysis of Chlorotalonil in Leek Using Mini-Luke Method Followed by GC-QqQ-MS/MS

CONTENTS

1. Aim and Scope 2
2. Short Description 2
3. Apparatus and Consumables 2
4. Chemicals 3
5. Procedure 3
   5.1 Sample Preparation 3
   5.2 Recovery Experiments for Method Validation 3
   5.3 Extraction 3
   5.4 Measurement 4
6. Evaluation of Results 5
7. Conclusion 5
8. References 5

Appendix I – Typical Chromatogram for Chlorothalonil 6
Example of calibration curve 6

1. Aim and Scope

This report describes a validation data for the analysis of chlorotalonil in leek. This matrix is considered of special difficulties as a consequence of high sulphur content compounds.

2. Short Description

The analysis of pesticide residues was performed by using Mini-Luke Method.

The homogeneous sample is extracted with acetone followed of partition with dichlorometane / petroleum ether (1:1). The mixture is centrifuged and an aliquot of the extract is concentrated to dryness. The residue is redissolved with ciclohexane / acetone (9:1) and injected in GC-MS/MS.

3. Apparatus and Consumables

- Sample processing equipment, e. g. Ditio Sama-K55 Food Processor.
- Ultra-Turrax, e.g. IKA T-25 digital.
- Centrifuge suitable for Teflon flask of 250 ml with screw caps, e.g.
- Haraeus. Labofuge GL and capable of achieving at least 4000 rpm.
- Graduate test tubes 10 to 100 ml.
- Automatic pipettes, suitable for handing volumes of 10 ml, 2 ml, 1 ml, 1 to 5 ml, 100 to 1000 µl, 10 to 100µl.
- Syringe, e.g. 2 ml, disposable syringes.
- Syringes filters, 0.45 µm pore size.
- Automatic dispenser, e.g. Ceramus Hirschmann Laborgerate of 10 to 60 ml.
- Volumetric flask of 10 ml and 100 ml.
- Concentration Workstation, e.g. TurvoVap LV Zymark
- Injection vials, 1.5 ml suitable for GC auto-sampler.

4. Chemicals

- Acetone, for GC residue analysis.
- Petroleum Ether, for GC residue analysis.
- Dichloramethane, for GC residue analysis.
- Ciclohexane, for GC residue analysis.

5. Procedure

5.1 Sample preparation

Sample was prepared according to the "*Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed" (Document No. SANCO/10684/2009)*.

Following this document, the leek 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

Commodities employed should not contain any of the pesticides analyzed. Organically grown samples are recommended for the analysis.

The validation method has been performed at two fortification levels (0.10 mg/Kg and 0.02 mg/Kg) and for each one five fortified samples and a blank have to be analyzed. In total eleven samples.

5.3 Extraction

1. Weigh 15 g ± 0.1 g of subsample in a wide-necked Teflon flask suitable for the centrifuge.
2. Sample fortification:
   - Low level concentration (0.02 mg/Kg)
   - High level concentration (0.10 mg/Kg)
3. Add 30 ml of acetone
4. Blend the sample with Ultra-turrax homogeneizer for 30 sec

5. Add 60 ml of petroleum ether – dichlorometane (1:1)
6. Blend the sample with Ultra-turrax homogeneizer for 30 sec
7. Centrifuge for 5 min at 4000 rpm
8. Transfer 10 ml extract into a test tube. Evaporate to dryness
9. Add 1 ml ciclohexane/acetone (9:1)
10. Vortex sample to mix it properly
11. Filter into an injection vial suitable for GC-MS/MS

5.4 Measurement

Instrumentation and Analytical Conditions for the GC/QqQ

- GC-MS/MS: Agilent 7890 Series
- Autosampler: Agilent 7683ª Injector and sample tray
- Inlet: Splitless
- Carrier gas: Helium
- Inlet pressure: 22.73 psi
- Inlet temperature: 250°C
- Injection volume: 1 µL
- Oven temperature program:

	Rate (°C/min)	Value	Hold Time (min)	Run Time (min)
Initial		70	2	2
Ramp 1	25	150	0	5.2
Ramp 2	3	200	0	21.867
Ramp 3	8	280	10	41.867

- Analytical column: Agilent J&W HP-5ms 30 m x 250 µm x 0.25 µm
- Retention time locking: chlorpyrifos methyl locked to 16.596 min
- Spectrometer: Agilent 7000B Series
- Source temperature: 280°C
- Quadrupole temperature: Q1 and Q" = 150°C
- Collision gas flows: Nitrogen at 1.5 mL/min, Helium at 25 ml/min

Mass transition for GC-MS/MS

PESTICIDE	R.T. (min)	TRANSITIONS
CHLOROTHALONYL	14.87	266.0 > 231.0 264.0 > 168.0 266.0 > 168.0

6. Evaluation of results

In the table below are shown the results for the mean recovery and RSD (Relative Standard Deviation) for the quantified pesticide at both levels.

PESTICIDE	LEVEL (mg/Kg)	R_1 (%)	R_2 (%)	R_3 (%)	R_4 (%)	R_5 (%)	MEAN (%)	RSD (%)
CHLOROTHALONIL	0.02	77	75	68	77	83	76	7
	0.10	75	76	72	71	63	72	7

The validation results are acceptable for the recovery and RSD values by the Document No. SANCO/10684/2009.

7. Conclusion

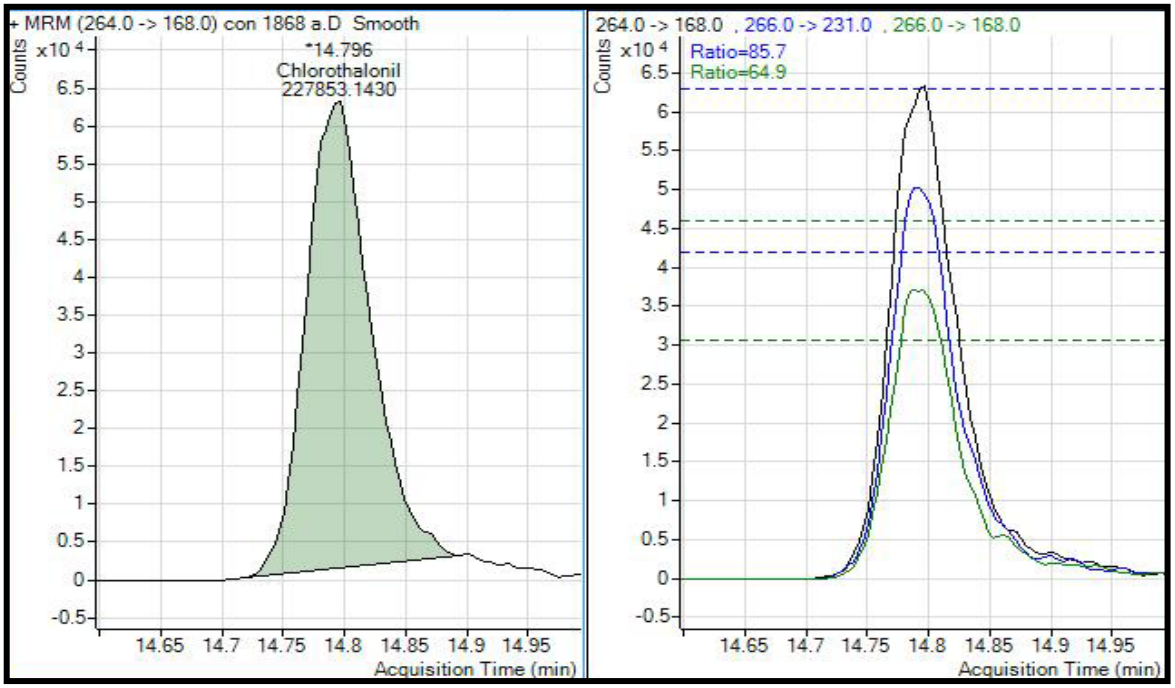
The results obtained are considered acceptable within the studied range. As this multiresidue method is used at the present time by a large number of laboratories within the European Pesticides Residues Monitoring Programme for fruit and vegetables, this survey can help to improve the analysis of chlorothalonil in difficult matrices as leek.

8. References

- Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed (Document No. SANCO/10684/2009)
- http://www.crl-pesticides.eu

APPENDIX I

TYPICAL CHROMATOGRAM OF CHLOROTHALONIL



EXAMPLE OF CALIBRATION CURVE