



EURL-FV
Multiresidue Method
using Mini-Luke followed
by GC-QqQ-MS/MS
for Fruits
and Vegetables

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	4
5.4 Measurement	4
5.5 Instrumentation and Analytical Conditions for the GC-MS/MS System	4
6. Calibration Curves	5
7. Evaluation of Results	6
8. Conclusion	6
9. References	6
Appendix I - Validation Results	7
Appendix II – Mass Transitions	8

1. Aim and Scope

This report describes a validation data for the analysis of 34 pesticides using a multiresidue method by GC-MS/MS in three representative matrices: grape, orange and tomato.

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 cyclohexane / 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. Heraeus. 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 μ l, 10 to 100 μ l.
- Syringe, e.g. 2 ml, disposable syringes.
- Syringes filters, 0.45 μ m 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.
- Dichloromethane, for GC residue analysis.
- Cyclohexane, for GC residue analysis.

5. Procedure

5.1 Sample preparation

Samples were 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 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 used for fortification were:

- Grape
- Orange
- Tomato

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

The validation method has to be performed at two fortification levels (0.50 mg/Kg and 0.05 mg/Kg). For each commodity five fortified samples and a blank have to be analyzed at each level. In total twelve samples per commodity.

5.3 Extraction

1. Weigh 15 g \pm 0.1 g of subsample in a wide-necked Teflon flask suitable for the centrifuge.
2. Sample fortification:
 - Low level concentration (0.05 mg/Kg)
 - High level concentration (0.50 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

Run the system in MRM-mode using the mass transitions presented in Appendix II.

Pesticides expressed according to the residue definition appear on the table with the index ^(RD).

5.5 Instrumentation and Analytical Conditions for the GC/QqQ

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

- Oven temperature program:

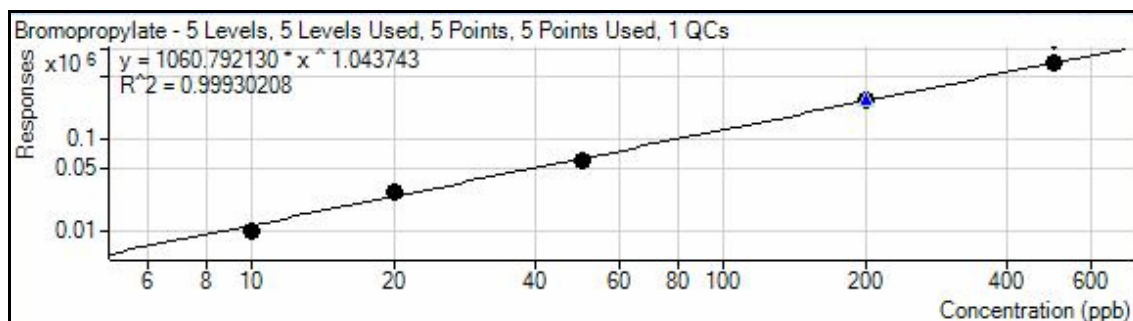
	Rate (°C/min)	Value	Hold Time (min)	Run Time (min)
Initial		70	2	2
Ramp 1	25	150	0	5.2
Ramp 2	3	200	0	21.867
Ramp 3	8	280	10	41.867

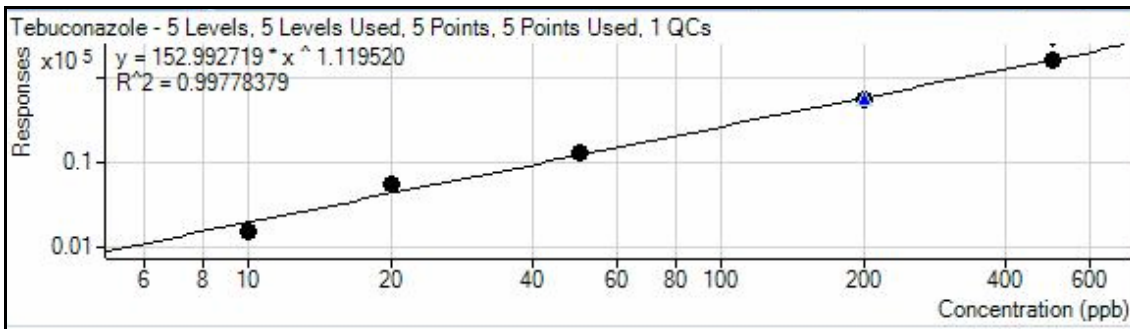
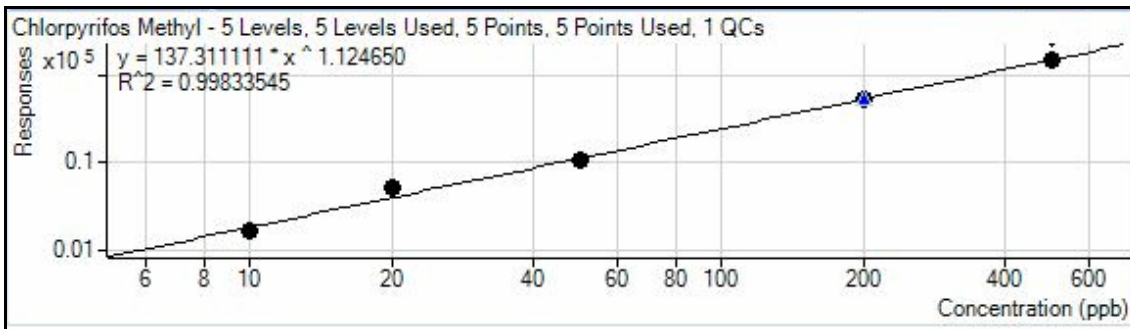
- Analytical column: Agilent J&W HP-5ms 30 m x 250 µm x 0.25 µm
- Retention time locking: chlorpyrifos-methyl locked to 16.596 min
- Spectrometer: Agilent 7000B Series
- Source temperature: 280°C
- Quadrupole temperature: Q1 and Q'' = 150°C
- Collision gas flows: Nitrogen at 1.5 mL/min, Helium at 25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

6. Calibration Curves

A calibration curve was calculated for each pesticide at four calibration levels; 0.01 µg/mL, 0.02 µg/mL, 0.05 µg/mL, 0.2 µg/mL and 0.5 µg/mL. The calibration curves were best fitted to a linear curve.

Figure 1: Examples of calibration curves





7. Evaluation of results

In the Appendix I are shown the results for the mean recovery and RSD (Relative Standard Deviation) for the quantified pesticides at both levels.

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

8. 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 pesticides mentioned above.

9. 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: VALIDATION RESULTS

PESTICIDE	MEAN% (0,05 mg/Kg)	RSD% (0,05 mg/Kg)	MEAN% (0,5 mg/Kg)	RSD% (0,5 mg/Kg)
BENALAXYL	118	8	93	8
BIFENTHRIN	111	5	95	7
BROMOPROPYLATE	112	4	97	7
BUPIRIMATE	82	8	84	7
CHLORFENAPYR	112	3	103	8
CHLORFENVINPHOS	115	3	105	9
CHLORPROPHAM	97	5	88	5
CHLORPYRIFOS	116	8	99	5
CHLORPYRIFOS-METHYL	112	4	102	8
CHLORTHIOPHOS	109	3	98	5
CYPERMETHRIN	117	4	101	5
DELTAMETHRIN	112	6	95	7
DICHOFLUANID	105	5	92	7
DIPHENYLAMINE	92	4	76	5
ENDOSULFAN ^(RD)	115	5	96	7
METALAXYL	112	4	85	8
O-PHENILPHENOL	85	5	82	5
PERMETHRIN	114	6	97	9
PHORATE	88	5	77	4
PHOSALONE	109	6	93	10
PIRIMICARB	86	13	85	10
PIRIMIPHOS-METHYL	88	4	98	6
PROFENOFOS	112	5	107	5
PROPICONAZOLE	103	5	99	6
PYRIDABEN	94	11	88	11
PYRIFENOX	89	6	88	6
PYRIPROXIFEN	85	9	104	9
QUINALPHOS	114	5	101	6
TEBUCONAZOLE	117	5	89	7
TEBUFENPYRAD	124	6	96	6
TERBUFOS	95	4	87	4
TOLYFLUANID	109	4	99	5
TRIFLURALIN	102	5	100	16
VINCLOZOLIN	112	4	97	11

APPENDIX II

MASS TRANSITION FOR GC - MS/MS

PESTICIDE	R.T. (min)	TRANSITION
BENALAXYL	26,74	266 > 148
		204 > 176
		148 > 105
BIFENTHRIN	28,84	181 > 166
		181 > 165
		181 > 153
BROMOPROPYLATE	28,62	341 > 185
		183 > 155
BUPIRIMATE	24,81	316 > 208
		273 > 193
		273 > 108
CHLORFENAPYR	25,23	247 > 227
		247 > 197
CHLORFENVINPHOS	21,55	267 > 159
		323 > 267
CHLORPROPHAM	11,04	213 > 171
		213 > 127
CHLORPYRIFOS	19,23	314 > 194
		197 > 169
		197 > 107
CHLORPYRIFOS METHYL	16,59	286 > 271
		286 > 093
CHLORTHIOPHOS	26,13	325 > 269
		269 > 205
CYPERMETHRIN	32,69	163 > 127
		181 > 152
		163 > 091
DELTAMETHRIN	36,00	253 > 093
		181 > 152
DICHLOFLUANID	18,41	332 > 167
		224 > 123
DIPHENYLAMINE	10,52	169 > 077
		168 > 051

PESTICIDE	R.T. (MIN)	TRANSITION
ENDOSULFAN α	22,64	241 > 206
		239 > 204
ENDOSULFAN β	25,16	241 > 206
		239 > 204
		195 > 159
ENDOSULFAN SULFATE	26,76	387 > 253
		387 > 217
METALAXYL	17,34	206 > 162
		206 > 132
O-PHENYLPHENOL	8,78	170 > 169
		170 > 141
		170 > 115
PERMETHRIN	31,37	183 > 168
		183 > 153
		183 > 115
PHORATE	11,96	260 > 93
		260 > 75
PHOSALONE	29,68	182 > 138
		182 > 111
PIRIMICARB	15,68	238 > 166
		166 > 96
PIRIMIPHOS METHYL	18,31	305 > 290
		305 > 180
		290 > 125
PROFENOFOS	23,90	337 > 267
		208 > 098
		208 > 063
PROPICONAZOLE	26,94	259 > 191
		259 > 173
		259 > 069
PYRIDABEN	31,53	147 > 117
		147 > 132
		309 > 147
PYRIFENOX	21,21	171 > 100
		171 > 136
		262 > 091
PYRIPROXYFEN	29,84	136 > 096
		136 > 078

PESTICIDE	R.T. (MIN)	TRANSITION
QUINALPHOS	29,86	146 > 118
		146 > 091
TEBUCONAZOLE	27,43	146 > 118
		146 > 091
		252 > 127
TEBUFENPYRAD	29,09	250 > 125
		333 > 276
TERBUFOS	13,80	333 > 171
		231 > 175
TOLYLFLUANID	2,25	231 > 129
		137 > 091
		137 > 065
TRIFLURALIN	11,64	306 > 264
		264 > 206
		264 > 160
VINCLOZOLIN	16,63	212 > 172
		212 > 145
		212 > 109