LC-QTOF-MS/MS method for the simultaneous full scan and MS/MS analysis of pesticides in fruit and vegetables

CONTENTS

1. Aim and Scope

This report describes the evaluation of LC-QTOF-MS for the simultaneous full scan and MS/MS analysis for the detection and identification of 125 pesticides in tomato, zucchini, orange, leek, onion and lettuce.

2. Procedure

Blank samples were examined to confirm the absence of target pesticides residues and used for preparing matrix-matched standards. All samples were spiked and diluted five times before LC-QTOF-MS/MS injection. Linearity and repeatability were evaluated in tomato and orange. Sensitivity, false negative and false positive detections were evaluated in all matrices. Quantitative capability of LC-QTOF-MS/MS working in simultaneous full scan MS/MS was also evaluated by five European Proficiency Test samples.

2.1. Sample extraction

The buffer citrate QuEChERS method was applied to the selected matrices.

2.2. Instrumentation and analytical conditions for the LC-QTOF-MS

2.2.1. Agilent 1290 HPLC

- Column: Agilent Eclipse Plus Rapid Resolution HD C18, 2.1 mm x 50 mm x1.8 µm
- Mobile phase A: acetonitrile 0.1% Formic Acid 5% ultrapure water
- Mobile phase B: 0.1% Formic acid in ultrapure water
- Flow rate: 0.3 mL/min
- Injection volume: 4 µL

Mobile phase gradient

Time [min]	Mobile phase A	Mobile phase B
0	20%	80%
2	20%	80%
15	100%	0%
17	100%	0%

Re-equilibration with initial mobile phase: 2.5 minutes.

2.2.2. Agilent 6550 LC-QTOF-MS

- 4GHz High Resolution Mode
- ESI source gas temperature: 160°C
- Gas flow: 14 L/min
- Nebuliser gas and collision gas: nitrogen
- Nebuliser gas pressure: 30 psi
- Sheath gas flow: 12 L/min
- Sheath gas temperature: 350 °C
- Ionisation mode: positive
- Capillary voltage: 4000 V
- OctapoleRFPeak 750V
- Fragmentor 250 V

Auto MS/MS settings

Full Scan

- Mass range 10-950 m/z
- Absolute threshold 500
- Acquisition rate 3 spectra/sec

MS/MS

- Inclusion list 125 pesticides
- Δ m/z 20 ppm
- Δ Rt 0.35 min
- Isolation width 1.3 m/z
- Mas range 65-750 m/z
- Acquisition rate 2 spectra/sec
- Absolute threshold 50
- Max 5 precursor per cicle
- Active exclusion
  - Excluded after 1 spectrum
  - Released after 0.7 min

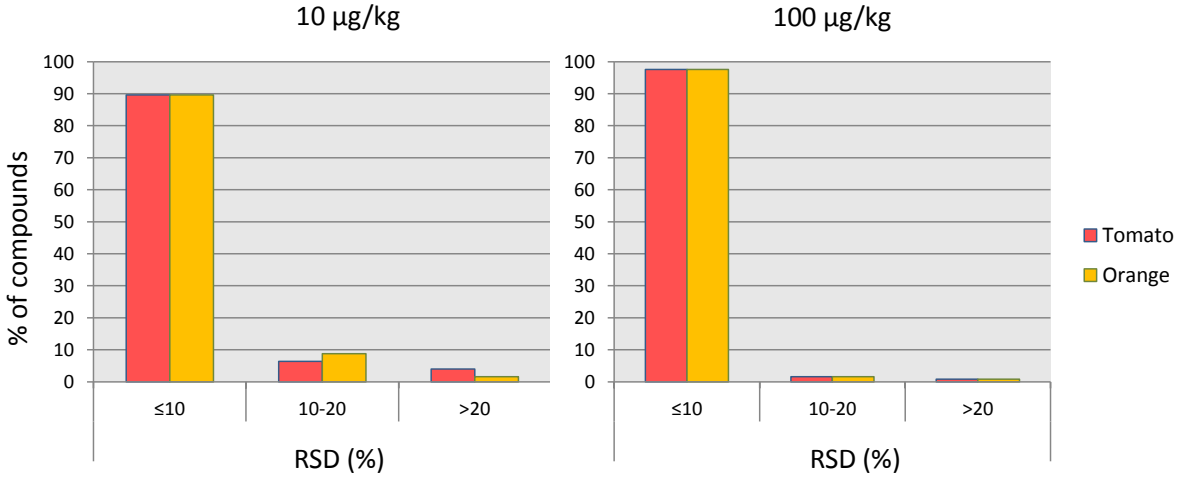
3. Method evaluation

3.1 Linearity

Linearity of the system was evaluated by assessing the signal responses of target analytes from matrix-matched calibration solutions by spiking blank extracts at eight concentration levels from 5 to 1000 µg kg^{-1}. In all cases the coefficient of determination (r^2) was higher than 0.99. Linearity ranges are summarized in Appendix I.

3.2 Repeatability

Repeatability of LC-QTOF-MS/MS in AutoMS/MS mode was evaluated by the injection of 5 replicates at 10 µg kg^{-1} and 100 µg kg^{-1} levels.

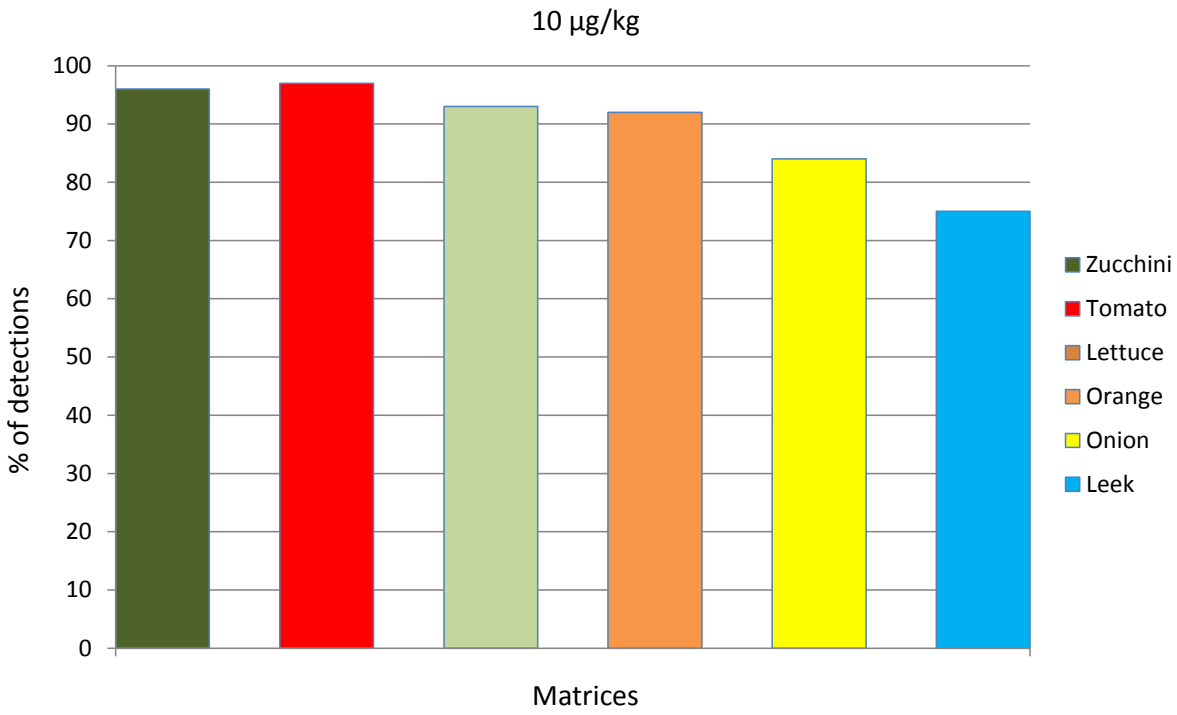


3.3 Reporting limits

Document SANCO/119457/2013 defines reporting limits as the lowest level that will be reported as absolute numbers. For easy matrices as tomato, reporting limit for 97% of pesticides was 10 µg kg^{-1}. For the rest of pesticides was between 20 and 50 µg kg^{-1}. In difficult matrices as orange reporting limit for 92% of pesticides was 10 µg kg^{-1}. For the rest of pesticides reporting limit was between 20 and 50 µg kg^{-1}

3.4 False negatives and positives determination

All blank matrices were spiked at 10 µg kg^{-1} level with the 125 pesticides and analysed by LC-QTOF-MS in AutoMS/MS mode in order to determine the percentage of detections and therefore false negative results. False positives were determined by the injection of the blank extracts by LC-QTOF-MS in AutoMS/MS mode



No false positive were identified in all the studied matrices.

4 Quantitation

Five European Proficiency Test in Fruit and Vegetables samples were quantified using the LC-QTOF-MS in AutoMS/MS mode. These samples correspond to the EUPT-FV 17 (Broccoli), EUPT-FV 16 (Pepper), EUPT-FV 15 (Potato), EUPT-FV 14 (Pear) and EUPT-FV 13 (Mandarin). Results are summarized in Appendix II.

APPENDIX I: LINEARITY RANGE

Pesticide	Linearity range (r^2>0.99)			
	Tomato		Orange	
	min	max	min	max
Acetamiprid	5	250	5	100
Aldicarb	5	100	5	50
Aldicarb sulfone	5	100	10	250
Aldicarb sulfoxide	100	750	-	-
Azoxystrobin	5	100	5	100
Bitertanol	20	1000	10	1000
Boscalid	10	250	5	250
Bromuconazole	10	250	10	1000
Bromuconazole	10	750	10	1000
Bupirimate	5	20	5	50
Buprofezin	5	20	5	50
Carbaryl	-	-	100	750
Carbendazim	5	20	5	100
Carbofuran	5	100	5	100
Chlorantraniliprole	10	750	10	250
Chlorfenvinphos	5	100	5	100
Clofentezine	10	500	20	250
Clothianidin	10	1000	20	750
Cyproconazole	10	500	5	500
Cyproconazole	5	250	5	250
Cyprodinil	5	20	5	50
Cyromazine	5	20	5	100
Demeton-S-methylsulfone	5	250	5	100
Diazinon	5	20	5	20
Dichlorvos	20	1000	20	1000
Dicrotophos	5	100	5	100
Diethofencarb	20	1000	20	1000
Difenoconazole	5	250	5	100
Difenoconazole	5	250	5	250
Diflubenzuron	10	1000	50	1000
Dimethoate	5	100	10	250
Dimethomorph	10	500	10	1000
Dimethomorph	5	1000	10	750
Diniconazole	5	100	5	100
Dodine	5	20	5	50
Epoxiconazole	5	100	5	100
Ethion	10	750	5	750
Ethirimol	5	20	5	50
Ethoprophos	5	100	5	100
Etofenprox	100	750	100	1000
Fenamidone	5	100	5	100

Fenamiphos	5	100	5	100
Fenamiphos sulfone	5	250	5	250
Fenamiphos sulfoxide	5	100	5	100
Fenarimol	5	750	5	500
Fenazaquin	5	20	5	50
Fenbuconazole	10	250	5	250
Fenhexamid	10	500	10	250
Fenpropimorph	5	20	5	50
Fenpyroximate	5	100	5	250
Fenthion	20	750	20	500
Fenthion sulfone	100	1000	100	750
Fenthion sulfoxide	5	100	5	100
Flonicamid	5	1000	5	500
Fluazifop	10	250	10	250
Flufenoxuron	10	500	10	750
Fluopyram	10	100	5	100
Fluquinconazole	5	1000	5	1000
Flusilazole	10	100	5	100
Flutriafol	5	100	5	250
Formetanate	5	20	5	50
Fosthiazate	5	100	20	250
Haloxyfop	5	1000	5	1000
Hexaconazole	5	100	5	100
Hexythiazox	20	1000	20	750
Imazalil	5	20	5	50
Imidacloprid	5	250	10	500
Indoxacarb	5	500	10	1000
Iprodione	10	750	50	1000
Iprovalicarb	5	100	10	100
Isocarbofos	5	100	5	100
Isofenphos-methyl	100	500	100	1000
Kresoxim-methyl	50	750	20	500
Linuron	10	250	5	100
Malathion	5	250	10	100
Mandipropamid	5	250	5	250
Mepanipyrin	5	20	5	20
Metalaxyl	5	100	5	100
Metconazole	5	100	5	100
Methidathion	5	50	5	100
Methiocarb	100	1000	100	1000
Methiocarb sulfone	250	1000	100	1000
Methiocarb sulfoxide	10	500	5	100
Methoxyfenozide	100	1000	20	250
Metobromuron	5	250	10	500
Monocrotophos	5	100	5	100
Myclobutanil	5	100	5	250

Nitenpyram	5	100	5	100
Omethoate	5	100	5	100
Oxamyl	5	50	5	100
Paclobutrazol	5	100	5	100
Penconazole	5	100	5	100
Pencycuron	5	100	5	100
Phenthoate	5	100	10	100
Phosalone	20	1000	20	1000
Phosmet	20	750	10	500
Pirimicarb	5	20	5	50
Pirimicarb desmethyl	5	20	5	50
Pirimiphos-methyl	5	20	5	50
Prochloraz	5	250	5	250
Profenofos	10	1000	10	500
Propamocarb	5	20	5	50
Propiconazole	5	100	5	250
Propiconazole	5	250	5	250
Propoxur	100	750	250	1000
Propyzamide	10	250	10	100
Pymetrozine	5	100	10	250
Pyraclostrobin	5	100	5	100
Pyridaben	5	100	5	250
Pyrimethanil	5	20	5	50
Pyriproxyfen	5	100	5	100
Quinoxyfen	5	100	5	100
Rotenone	5	250	20	500
Spinosyn A	5	100	10	250
Spinosyn D	5	750	10	750
Spirodiclofen	20	750	100	1000
Spiromesifen	50	750	250	1000
Spiroxamine	5	20	5	50
Tebuconazole	5	100	5	100
Tebufenozide	100	1000	50	1000
Tebufenpyrad	5	250	10	250
Terbuthylazine	5	20	5	100
Tetraconazole	5	100	5	250
Thiabendazole	5	20	5	100
Thiacloprid	5	250	5	250
Thiamethoxam	5	50	5	100
Thiodicarb	10	750	10	250
Triazophos	5	100	5	100
Trichlorfon	50	1000	50	1000
Trifloxystrobin	10	100	5	100
Triflumuron	10	250	20	1000
Triticonazole	10	250	5	250
Zoxamide	10	100	5	100

APPENDIX II: Quantitation

EUPT-FV-17 Broccoli			
Pesticide	Measured concentration mg/kg	Median from PT mg/kg	Relative error %
Bupirimate	0.171	0.165	4
Carbendazim	0.527	0.512	3
Diazinon	0.080	0.071	13
Difenoconazole	0.597	0.530	13
Diflubenzuron	0.392	0.319	23
Methoxyfenocide	0.302	0.349	-13
Spinosad	0.067	0.051	31
Thiabendazole	1.782	1.900	-6
Trifloxistrobin	0.517	0.466	11

EUPT-FV-16 Pepper			
Pesticide	Measured concentration mg/kg	Median from PT mg/kg	Relative error %
Acetamiprid	0.676	0.632	7
Buprofezin	0.512	0.467	10
Cyprodinyl	0.650	0.591	10
Diazinon	0.098	0.084	17
Difenoconazole	1.012	0.953	6
Fenamiphos	6.985	7.385	-5
Fenamiphos sulfone	0.122	0.110	11
Fenamiphos sulfoxide	7.031	6.635	6
Fenhexamid	0.947	0.861	10
Methoxyfenocide	0.175	0.165	6
Pirimicarb	0.731	0.731	0
Pyridaben	0.155	0.151	3
Spinosad	0.105	0.094	12
Tetraconazole	0.098	0.104	-6

EUPT-FV-15 Potato

Pesticide	Measured concentration mg/kg	Median from PT mg/kg	Relative error %
Diazinon	0.242	0.195	24
Fosthiazate	0.100	0.080	24
Iprovalicarb	0.091	0.090	1
Linuron	0.096	0.098	-2
Methiocarb	0.152	0.136	12
Spiridiclofen	0.490	0.444	10
Thiabendazole	1.865	1.710	9
Thiacloprid	0.403	0.338	19

EUPT-FV-14 Pear

Pesticide	Measured concentration mg/kg	Median from PT mg/kg	Relative error %
Boscalid	0.217	0.177	23
Cyprodinil	0.254	0.247	3
Diazinon	0.057	0.053	8
Flufenoxuron	0.385	0.491	-22
Indoxacarb	0.081	0.083	-2
Methoxyfenocide	0.158	0.154	3
Pyraclostrobin	0.151	0.172	-12
Pyrimethanyl	0.117	0.107	9
Spirodiclofen	0.106	0.111	-5
Thiabendazole	0.514	0.461	11
Thiacloprid	0.034	0.030	13
Triflumuron	0.551	0.455	21

EUPT-FV-13 Mandarin

Pesticide	Measured concentration mg/kg	Median from PT mg/kg	Relative error %
Carbendazim	1.260	1.250	1
Diazinon	0.209	0.189	11
Imazalil	1.236	1.300	-5
Indoxacarb	0.943	0.792	19
Malathion	0.489	0.381	28
Methidation	0.902	0.730	24
Oxamyl	0.131	0.132	-1
Phosalone	0.262	0.280	-7
Prochloraz	0.261	0.301	-13
Pyriproxifen	0.385	0.443	-13
Spinosad	0.650	0.608	7
Thiabendazole	0.888	0.810	10