High-Resolution Accuracy Mass (HRAMS) methods for the screening of pesticides not approved under Reg. (EC) Nº 1107/2009, using exact mass libraries and databases.

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1. Aim and scope

Constant positive findings of non-authorized pesticides in the European Union or authorised substances with unauthorised use brings out the need to include some of these low-frequency pesticides in routine analysis. However, due to their low frequency, for an effective handling it is advisable to include them in HRAMS screening methods. With this aim the use of an exact mass library for GC-HRAMS and a database for LC-HRAMS have been evaluated in order to apply them in the aforementioned screening methods.

2. Procedure

2.1 GC-TOF-MS screening

Orange and tomato samples were extracted using the QuEChERS citrate method. The final extracts were evaporated, reconstituted with ethyl acetate and spiked at a concentration level of 10 µg/kg and 50 µg/kg with a mix of 60 pesticides (Table 1). The pesticides were selected in the study for the status of not approved under Reg. (EC) N° 1107/2009 [1]. Then extracts were analysed using GC-TOF-MS system.

The screening of non-authorized pesticides by GC-TOF-MS was performed using a spectral exact mass library of the 60 pesticides belonging from the Agilent Mass Hunter GC/Q-TOF Pesticide PCDL (Personal Compound Database and Library). The PCDL contains high-resolution accurate mass spectra and reference retention time for the retention time locked chromatographic method used in this study.

Data were processed in two ways:

- Using Agilent MassHunter Unknown Analysis B.10. Peak detection was performed SureMass with a filter of 10000 counts. Identification was done

by comparing exact mass spectra obtained with EI ionization with the PCDL containing the 60 non- authorized pesticides. Comparison with the exact mass library was carried out setting a maximum retention time deviation of ± 1 min and a minimum match factor score of 40.

- Using Agilent MassHunter Quantitative Analysis Software B. 10.0. Two most abundant and/or selective ions were selected from de PCL and included in the quantitative software. The criteria for identification were two ions in the extracted ion fully overlapped, with a mass accuracy ≤5 ppm, signal to noise ratio ≥3 and retention time ± 0.1 minutes.

2.2 LC-QOrbitrap-MS screening

Orange and tomato samples were extracted using the QuEChERS citrate method. The final extracts were evaporated, reconstituted with acetonitrile and spiked at a concentration level of 10 µg/kg and 50 µg/kg with a mix of 66 pesticides (Table 2). Before the injection, a five-fold dilution with water was applied. The pesticides were selected in the study for the status of not approved under Reg. (EC) N° 1107/2009 [1]. Then extracts were analysed using LC-QOrbitrap system.

The screening of non-authorized pesticides by LC-QOrbitrap was performed using a data base of the 66 pesticides.

Data were processed with TraceFinder 4.1. Mass tolerance for both MS and MS2 was 5 ppm. Intensity threshold in MS was set at 5 000, intensity threshold in MS2 set at 1 000.

3. Instrumentation and analytical conditions for the GC-TOF-MS

Agilent 7890 A: Settings for gas chromatography:

- Injection mode: solvent vent
- Injection volume: 5 µL
- Ultra-Inert liner with a glass wool frit
- Carrier gas: Helium
- Two online columns: HP-5MSUI (15m x 0.250mm x 0.250µm)
- Oven gradient:

Rate (°C/min)	Time (min)	Hold Time (min)	T (°C)
	60	1	1
40	170	0	3.75
10	310	3	20.75

- Transfer line temperature: 280°C
- Retention time locked with constant flow
- Backflushing at 310 °C for 2 min

Agilent 7250 GC/Q-TOF

- EI ion source operation at 70 eV
- 4GHz High Resolution Mode
- Ion source temperature 280 °C
- Acquisition
- Acquisition: Full scan m/z range of 60-500 amu
- Acquisition rate: 3 spectra/s

4. Instrumentation and analytical conditions for the LC-QOrbitrap_MS (QExactive)

Chromatography

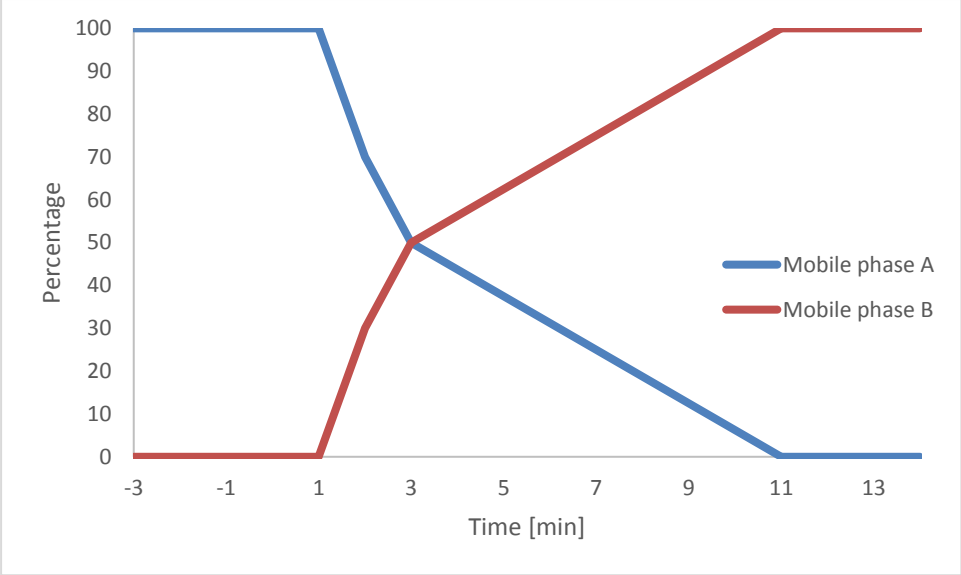
Mobil phase:

A: 98% H2O 2% MeOH 5mM HCOONH4 0.1% HCOOH

B: 98% MeOH 2% H2O 5mM HCOONH4 0.1% HCOOH

Flow: 0.35 mL/min

Gradient time: 14 min + 3 min reequilibration



Column: Phenomenex Luna, 100 mm x 2 mm x 2.6 µm

Column temperature: 30°C

Injection volume: 10 µL

Mass spectrometry

ESI parameters:

Polarity: positive

Sheath gas flow rate: 40

Aux gas flow rate: 5

Sweep gas flow rate: 1

Spray voltage: 3 000 V

Capillary temperature: 280 °C

S-lens RF level: 44

Aux gas heater temperature: 350 °C

Acquisition in Full MS:

Resolution: 70 000 at m/z 200

Scan range: m/z 100 – m/z 1000

AGC target: 1e6

Maximum IT: auto

Acquisition in AIF MS2:

Resolution: 70 000 at m/z 200

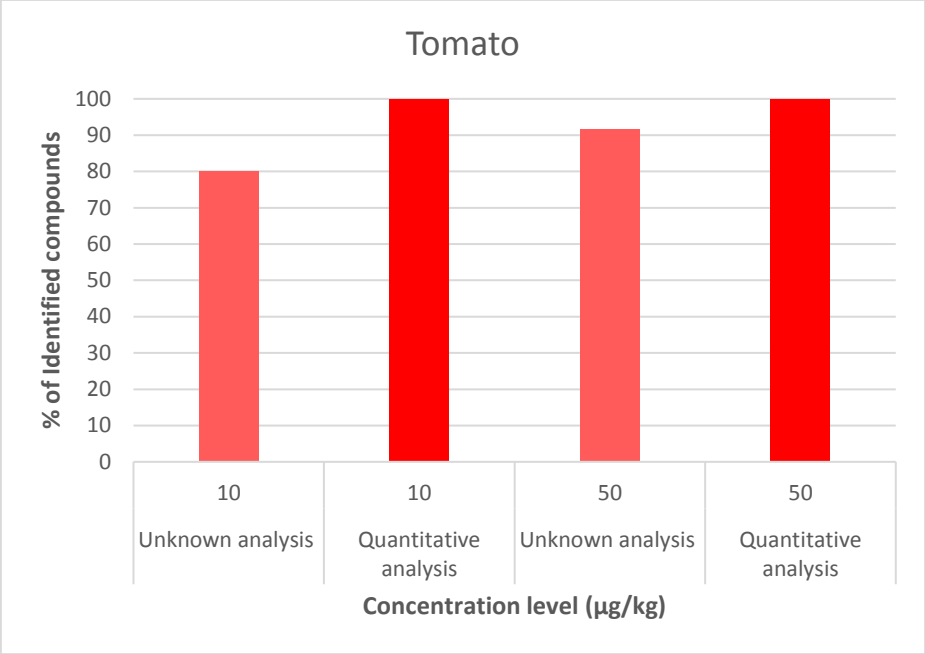
Scan range: m/z 66.7 – m/z 1000

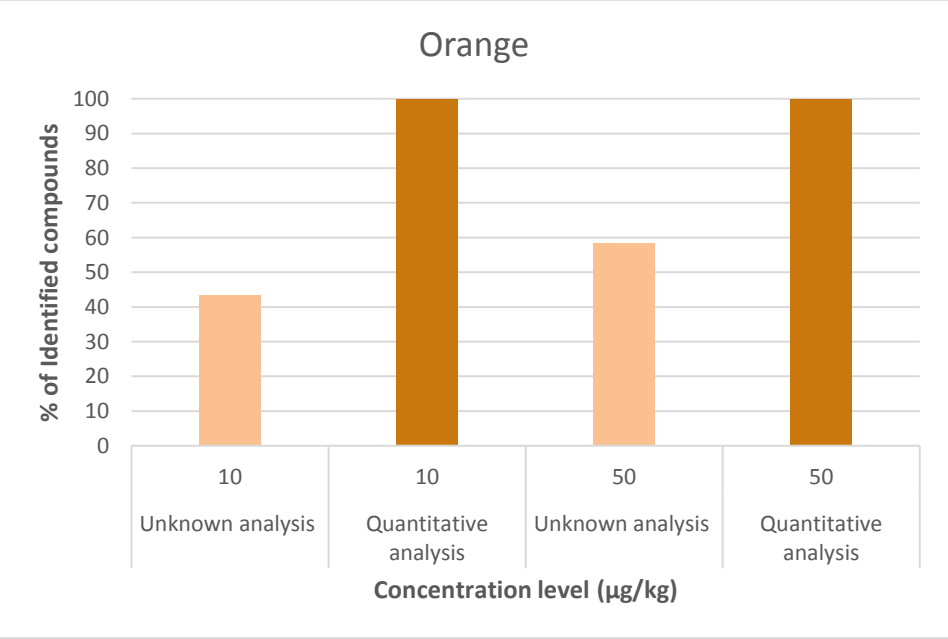
Colision energy: 25 eV

AGC target: 3e6

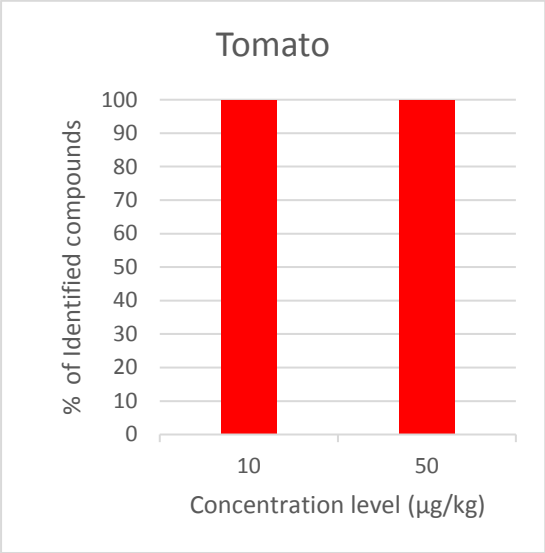
Maximum IT: auto

5. GC-TOF-MS results





6. LC-Orbitrap-MS results



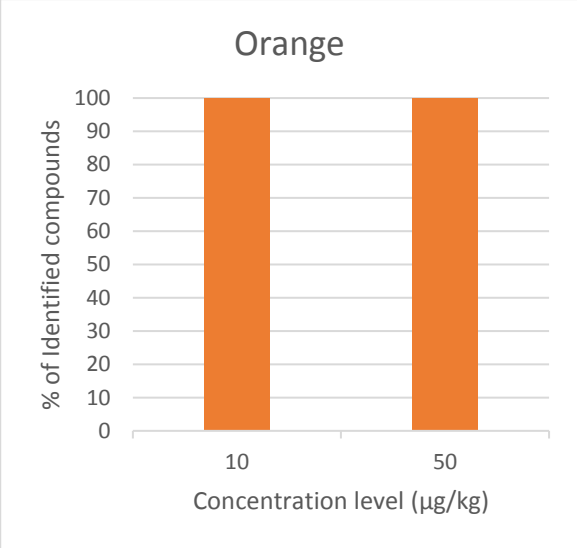


Table I. List of studied pesticides using GC-TOF-MS

Pesticides	Multiannual control programme of the Union, 2019 [2]	Analysis Technique
Alachlor	Not included	GC-TOF
Ametryne (Ametrex)	Not included	GC-TOF
Atrazine	Not included	GC-TOF
Biphenyl	Included	GC-TOF
Bromopropylate	Included	GC-TOF
Butralin (Sutralin)	Not included	GC-TOF
Butylate (Sutan)	Not included	GC-TOF
Carbophenothion	Not included	GC-TOF
Chlorfenapyr	Included	GC-TOF

Chlorobenzilate	Not included	GC-TOF
Chlozolinate	Not included	GC-TOF
Cyfluthrin	Included	GC-TOF
Diazinon (Dimpylate)	Included	GC-TOF
Dichlofluanid	Not included	GC-TOF
Dicofol	Included	GC-TOF
Dieldrin	Included	GC-TOF
Dimethenamid (SAN 582H)	Not included	GC-TOF
Endrin	Not included	GC-TOF
EPN / Tsumaphos	Not included	GC-TOF
Ethoprophos (Ethoprop)	Not included	GC-TOF
Fenamidone	Included	GC-TOF
Fenarimol	Included	GC-TOF
Fenitrothion	Included	GC-TOF
Fenthion	Included	GC-TOF
Fenvalerate	Included	GC-TOF
Fluacrypyrim	Not included	GC-TOF
Fonofos	Not included	GC-TOF
Formothion	Not included	GC-TOF
Heptachlor	Included	GC-TOF
Heptenophos	Not included	GC-TOF
Hexaconazole	Included	GC-TOF
Isazofos (Miral)	Not included	GC-TOF
Isoprothiolane	Not included	GC-TOF
Mecarbam	Not included	GC-TOF
Methidathion	Included	GC-TOF
Metolachlor	Not included	GC-TOF
Mevinphos (Phosdrin)	Not included	GC-TOF
Molinate	Not included	GC-TOF

Pebulate	Not included	GC-TOF
Phenothrin	Not included	GC-TOF
Phenthoate (Fenthoate)	Not included	GC-TOF
Phorate	Not included	GC-TOF
Profenofos	Included	GC-TOF
Propaphos	Not included	GC-TOF
Propazine	Not included	GC-TOF
Propiconazole	Included	GC-TOF
Pyrifenox	Not included	GC-TOF
Quinalphos (Diethquinalphione)	Not included	GC-TOF
Quinoxyfen	Included	GC-TOF
Sulfotep	Not included	GC-TOF
Sulprofos	Not included	GC-TOF
Terbufos	Not included	GC-TOF
Terbumeton	Not included	GC-TOF
Terbutryn	Not included	GC-TOF
Tolylfluanide	Not included	GC-TOF
Triadimefon	Included	GC-TOF
Trifluralin	Not included	GC-TOF
Vinclozolin	Included	GC-TOF
Coumaphos	Not included	GC-TOF
Ofurace	Not included	GC-TOF

Table 2. List of studied pesticides using LC-Orbitrap-MS

Pesticides	Multiannual control programme of the Union, 2019 [2]	Analysis Technique
Acephate	Included	LC-Orbitrap
Alachlor	Not included	LC-Orbitrap
Atrazine	Not included	LC-Orbitrap
Azinphos-methyl	Included	LC-Orbitrap
Bendiocarb	Not included	LC-Orbitrap
Bitertanol	Included	LC-Orbitrap
Bromacil	Not included	LC-Orbitrap
Carbaryl	Included	LC-Orbitrap
Carbendazim	Included	LC-Orbitrap
Chlorfluazuron	Not included	LC-Orbitrap
Chloroxuron	Not included	LC-Orbitrap
Coumaphos	Not included	LC-Orbitrap
Demeton-S-methyl	Not included	LC-Orbitrap
Diazinon	Included	LC-Orbitrap
Dicrotophos	Not included	LC-Orbitrap
Difenoxuron	Not included	LC-Orbitrap
Edifenphos	Not included	LC-Orbitrap
Ethiofencarb	Not included	LC-Orbitrap
Ethiprole	Not included	LC-Orbitrap
Ethirimol	Included	LC-Orbitrap
Ethoprophos	Not included	LC-Orbitrap
Fenamidone	Included	LC-Orbitrap
Fenarimol	Included	LC-Orbitrap

Fenobucarb	Not included	LC-Orbitrap
Fenthion	Included	LC-Orbitrap
Fluacrypyrim	Not included	LC-Orbitrap
Flufenoxuron	Included	LC-Orbitrap
Flusilazole	Included	LC-Orbitrap
Hexaconazole	Included	LC-Orbitrap
Isoprothiolane	Included	LC-Orbitrap
Isoproturon	Not included	LC-Orbitrap
Linuron	Included	LC-Orbitrap
Methamidophos	Included	LC-Orbitrap
Methidathion	Included	LC-Orbitrap
Metolachlor	Not included	LC-Orbitrap
Metolcarb	Not included	LC-Orbitrap
Monocrotophos	Included	LC-Orbitrap
Monolinuron	Not included	LC-Orbitrap
Monuron	Not included	LC-Orbitrap
Neburon	Not included	LC-Orbitrap
Novaluron	Not included	LC-Orbitrap
Omethoate	Included	LC-Orbitrap
Oxadiargyl	Not included	LC-Orbitrap
Oxadixyl	Included	LC-Orbitrap
Oxasulfuron	Not included	LC-Orbitrap
Phenthoate	Not included	LC-Orbitrap
Phosalone	Not included	LC-Orbitrap
Phoxim	Not included	LC-Orbitrap
Profenofos	Included	LC-Orbitrap
Propargite	Included	LC-Orbitrap
Propazine	Not included	LC-Orbitrap
Propiconazole	Included	LC-Orbitrap

Propoxur	Not included	LC-Orbitrap
Pymetrozine	Included	LC-Orbitrap
Pyridaphenthion	Not included	LC-Orbitrap
Quinalphos	Not included	LC-Orbitrap
Quinoclamine	Not included	LC-Orbitrap
Quinoxyfen	Included	LC-Orbitrap
Quizalofop-ethyl	Not included	LC-Orbitrap
Rotenone	Not included	LC-Orbitrap
Simazine	Not included	LC-Orbitrap
Thiobencarb	Not included	LC-Orbitrap
Tolfenpyrad	Not included	LC-Orbitrap
Triazophos	Included	LC-Orbitrap
Trichlorfon	Not included	LC-Orbitrap
Tricyclazole	Included	LC-Orbitrap

References

[1] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

[2] Regulation (EU) 2019/533 of 28 March 2019 concerning a coordinated multiannual control programme of the Union for 2020, 2021 and 2022 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin