Performance evaluation of UHPLC-Q-TOF HRMS for pesticide residues analysis in food samples

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INTRODUCTION

Our laboratory acquired a Bruker UHPLC-Q-TOF HRMS-Impact II instrument, with a resolving power up to 50.000 (at m/z 800). A critical evaluation of the merits of UHPLC-Q-TOF compared to our Waters UHPLC-triple quad MS/MS (XEVO-TQ-S) was performed as to the achievable detection levels and false-positives/false-negatives rates, for various settings of the UHPLC-Q-TOF (mass accuracy error, retention time window, required isotopic ions and/or fragment ions, ion ratios). A method validation has been carried out for lettuce and orange and a comparison of pesticide concentrations in routine samples measured by LC-MS/MS and LC-HRMS will be presented as well. An extensive evaluation of the instrument performance has been done by measuring 20 (difficult) matrices, spiked at 10 and 50 µg/kg.

METHODS

A 15 g sample is extracted in 250-mL a PTFE-tube with 20 mL acetone (30 sec, Polytron homogenizer), followed by partitioning (30 sec) with 20 mL petroleum ether, 10 mL CH_2Cl_2 and 15 g Na_2SO_4 (salting-out effect assuring good recoveries for polar pesticides; NL-method). After centrifugation (5 min at 3500 rpm), an aliquot (3,33 mL) of the extract is evaporated to dryness and the residue is redissolved in 1 mL methanol (matrix concentration, 1 g/mL). A volume of 2 µL is injected into the UHPLC-Q-TOF system.

Experimental UHPLC conditions:

UHPLC-system:	Dionex Ultimate 3000, binary gradient
UHPLC-column:	Dionex Acclaim RSLC 120 C18 (2.2 µm, 120 Å), 100 x 2.1 mm
Eluent A:	Water: Methanol (90:10) + 5 mM ammonium formate + 0.01% formic acid
Eluent B:	Methanol + 5 mM ammonium formate + 0.01% formic acid

UHPLC-Gradient

Step	Time (min.)	Flow (mL/min.)	Eluent B (%)
1	0	0.2	1.0
2	0	0.2	1.0
3	0.1		1.0
4	1.0	0.2	1.0
5	3.0		39.0
6	14.0	0.4	99.9
7	16.1	0.48	99.9
8	16.1	0.48	1.0
9	19.0	0.48	1.0
10	19.1	0.2	1.0

Bruker Impact II Q-TOF

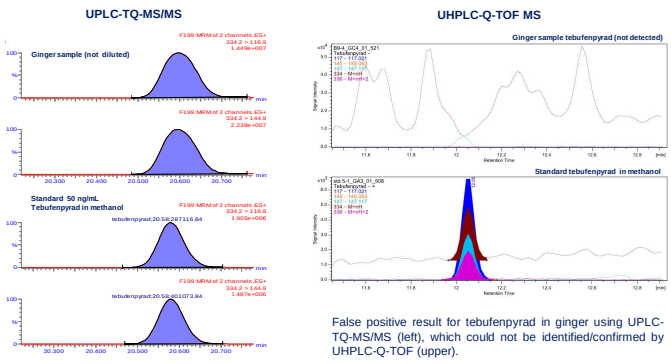
Q-TOF parameters, ESI+ mode, mass range m/z 30 -1000

Source:		**Collision Cell**	
End plate offset (V, nA)	500, 54	Collision Energy	18 and 32 eV
Capillary (V, nA)	2500, 150	Pre pulse storage	5 µs
Nebulizer pressure (bar)	2	Stepping	Basic
Dry gas flow rate (L/min.)	8		
Dry temp (°C)	200		From To
		Collision Rt (Vpp)	250 1000
Tune:		Transfer time (µs)	25 70
Transfer Funnel 1 (Vpp)	150	Timing (%)	50 50
Transfer Funnel 2 (Vpp)	150		
CID energy (eV)	0	**MS/MS**	
Hexapole RF (Vpp)	30	Precursor ions	5
		No of precursors	3
Quadrupole		Cycle time (s)	3
Ion energy (eV)			
Low mass (m/z)	70	Scan rate (Hz)	2

The Q-TOF is calibrated within each run, using sodium formate clusters. An internal lock mass is used (methylstearate).

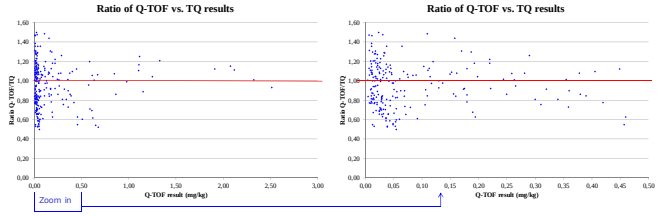
Alternating acquisition in Full Scan mode and Broadband Collision-Induced Dissociation (bbCID) mode (MS/MS fragmentation) is applied. Data processing is performed post-run with PesticideScreener 2.0 (database with ± 860 pesticides) and TASQ 1.4 software for unequivocal identification and accurate quantification.

COMPARISON of Q-TOF MS with TQ-MS/MS



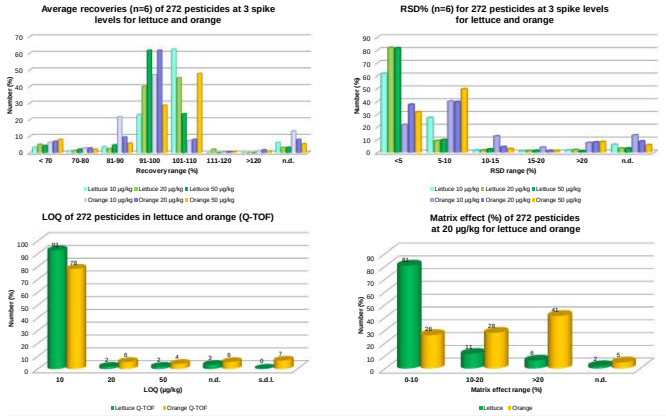
False positive result for tebufenpyrad in ginger using UPLC-TQ-MS/MS (left), which could not be identified/confirmed by UHPLC-Q-TOF (upper).

Difference between quantitative results of UHPLC-Q-TOF and UPLC-TQ-MS/MS from routine samples



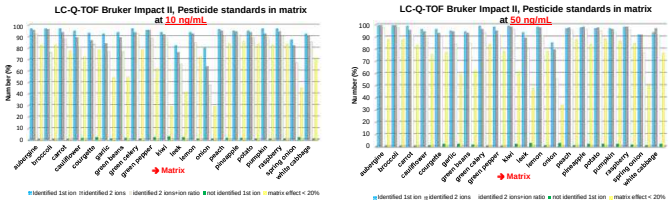
QUANTITATIVE RESULTS – Method Validation

- Validation of the method was carried out for 272 representative pesticides from different pesticide classes in the matrices lettuce and orange. Recovery studies were performed with spiking levels of 10, 20 and 50 ug/kg. Trueness (as % recovery, n=6), and precision (as repeatability %RSD, n=6), linear dynamic range of the calibration curve, instrument LODs, matrix effects and method-LOQs were determined.
- The majority of analytes (93% for lettuce, 78% for orange) met the EU DG SANTE method validation criteria (i.e. average recoveries in the range 70-120%, with RSD <20%, mass error < 5 ppm, Δ Rt < 0.1 min.) at the lowest spike level (10 µg/kg).
- Validated method-LOQs are 10 µg/kg for the majority of analytes.
- Matrix effects are not (lettuce) to slightly (orange) significant.

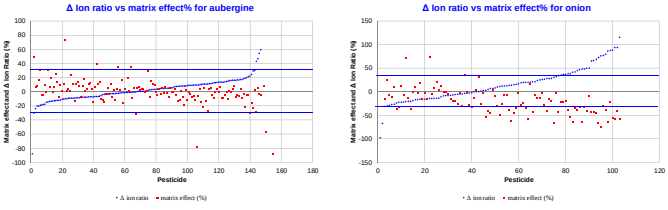


RESULTS – 20 different matrices

% of pesticides meeting identification criteria for 20 matrices



Identification criteria:
Mass accuracy: < 5 ppm (<1 mDa for m/z < 200 Da); Δ Ion Ratio ≤ 30% (indicative); Rt ≤ 0.1 min.



The majority of the 155 pesticides investigated in 20 different matrices meet the identification criteria.
The mass accuracy is fulfilled for > 90 % of the pesticides for most matrices. For the well known "difficult matrices" like onion and leek, this percentage is somewhat lower. This lower percentage is also caused by the fact that, due to the sometimes high matrix effects for these matrices, more pesticides could not be detected at the 10 ng/mL (10 µg/kg) level.
The ion-ratio for the majority of the compounds is ≤ 30%.
The criterion of the Rt difference is met for all pesticides investigated.
From the two lower figures it can be seen, that sometimes matrix effects / interferences occur, which influence the Ion Ratio.

CONCLUSIONS

- The majority of the 272 analytes met the EU SANTE method validation criteria in the matrices lettuce and orange (i.e. average recoveries 70-120%, RSD <20%) at the tested spike levels, with a mass error ≤ 5 ppm (or ≤ 1 mDa for ions with m/z <200), ΔRt ≤ 0.1 min and Δ ion-ratio ≤ 30% (indicative criterion). Validated LOQs of 10 µg/kg were easily achieved for most analytes.
- Calibration curves (1-50 ng/mL) were linear (r^2 >0.98) for almost all detected analytes.
- The instrument LOD for the majority of analytes is well below 10 ng/mL (for a 2 µL injection).
- The validated method has been applied successfully for fruit and vegetables and other difficult matrices, like vine leaves, tea and spices, screening for more than 860 pesticides.
- The UHPLC-Q-TOF (Impact II) is a factor of 5-10 less sensitive than the high-end sensitive Xevo TQ-S MS/MS TQ, comparing absolute amount injected on-column.