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Matrix interferences evaluation employing GC and LC coupled to triple quadrupole tandem mass spectrometry

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INTRODUCTION

Gas and liquid chromatography coupled to triple quadrupole tandem mass spectrometry are two very powerful tools commonly employed for the analysis of pesticide residues in fruit and vegetables. However, due to the big quantity and complexity of the commodities different responses are produced, making difficult to identify/quantify a compound.

To get a clear evaluation twenty five different matrices were purchased from local markets in Almería, extracted with four commonly used extraction methods (citrate QuEChERS with/without clean-up, ethyl acetate extraction method [1], and Dutch mini-Luke method [2]), and evaluated with both GC-QqQ-MS/MS and LC-QqQ-MS/MS. To consider a present peak as a possible interference, the criteria of ± 0.2 min over the retention time of the standard and a signal-to-noise ratio higher than 3 must be taken, according to the criteria in DG-SANCO.

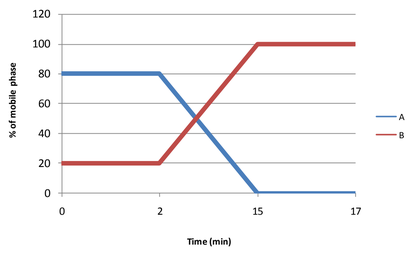
LC-QqQ-MS/MS

System:

UPLC 1290 coupled to a 6490 Triple Quad LC/MS (Agilent)

LC Parameters:

- Injection volume: 5 µL
- Flow rate: 300 µL/min
- Column: Zorbax Eclipse PlusC8 1.8 µm, 2.1x100mm
- Total run time: 17 + 2.5 min (Post Time)
- Mobile Phases and gradient :
  - o A → H2O 0.1% formic acid
  - o B → AcN 0.1% formic acid and 5% water



MS Parameters:

- Dynamic MRM
- Positive and Negative mode
- ESI

Extraction Methods

Citrate QuEChERS

10 g of Sample + 10 ml of AcN

Shake 4 min automatically

4 g $MgSO_4$ + 1 g NaCl + 1 g $NaAc{\cdot}2H_2O$ +0.5 g $NaAc{\cdot}1.5H_2O$

Shake 4 min automatically

Centrifuge 5 minutes at 3700 rpm

Take 5 mL + 750 mg MgSO4 + 125 mg PSA

Centrifuge 5 minutes at 3700 rpm

Analysis

Citrate QuEChERS without Clean-up

10 g of Sample + 10 ml of AcN

Shake 4 min automatically

4 g $MgSO_4$ + 1 g NaCl + 1 g $NaAc{\cdot}2H_2O$ +0.5 g $NaAc{\cdot}1.5H_2O$

Shake 4 min automatically

Centrifuge 5 minutes at 3700 rpm

Analysis

Ethyl Acetate

10 g of Sample + 10 ml of EtOAc

1.5 g NaCl + 8 g $MgSO_4$

Shake 15 min automatically

Centrifuge 5 minutes at 3700 rpm

Analysis

Dutch mini-Luke

15 g of Sample + 20 ml of Acetone + 15 g Na_2SO_4

Mix 30 sec by Turrax

Add 20 ml PE + 10 ml DCM

Mix 30 sec by Turrax

Centrifuge 3 minutes at 3300 rpm

Analysis

Amount Injected:

- o **LC: 0.2 g of sample/ml of extract**
- o **GC: 1 g of sample/ml of extract**

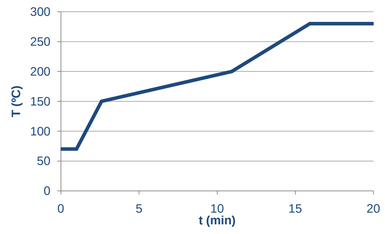
GC-QqQ-MS/MS

System:

7890 GC System coupled to a 7000 GC/MS Triple Quad (Agilent)

GC Parameters:

- Injection volume: 2 µL
- Splitless mode
- Column: HP-5MSUI (15 m x 250 µm x 0.25 µm)
- Carrier gas: Helium
- Constant pressure: 15.35 psi
- Total run time: 20 + 3 min (Post Run)
- Oven temperature program:



MS Parameters:

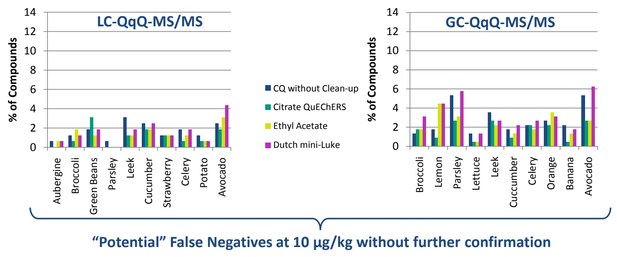
- MRM
- Positive mode
- Electron Impact

Interferences in Selected Reaction Monitoring

In the case of LC-QqQ-MS/MS, around 160 compounds have been monitored. There are no differences between the methods tested, never exceeding 3 % of interferences, in the ± 0.2 min retention time range. The matrices presenting the larger number of interferences were green beans, leek and celery.

On the other hand, the study developed by GC-QqQ-MS/MS has been carried out monitoring over 220 pesticides. Highest number of interferences can be seen for ethyl acetate extraction method and Dutch mini-Luke method, due to the solvents employed for these extractions are more apolar (ethyl acetate and acetone, respectively), but in any case interferences exceed 15 % (avocado with ethyl acetate method), in the ± 0.2 min retention time range. In all methods the highest number of interferences was obtained for avocado, nevertheless other complex matrices detected were parsley and cucumber.

The ten matrices presenting highest percentage of interferences were selected, and their extracts were spiked to study false negatives generated by a matrix peak interference. To develop this study, only interferences overlapping with compound peaks, and so making the difference in ion ratio > 30 %, were taken into account. Again, higher number of false negatives at 10 µg/kg can be observed in results provided by GC-QqQ-MS/MS due to the ionization source employed in GC (EI) generate less selective transitions. To decrease the percentage of false negatives two options can be considered: try to select a third more selective transition and/or confirm with other chromatographic technique.



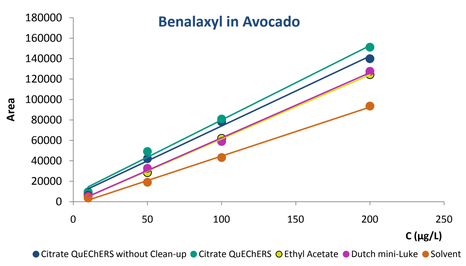
"Potential" False Negatives at 10 µg/kg without further confirmation

Linearity and Matrix Effect

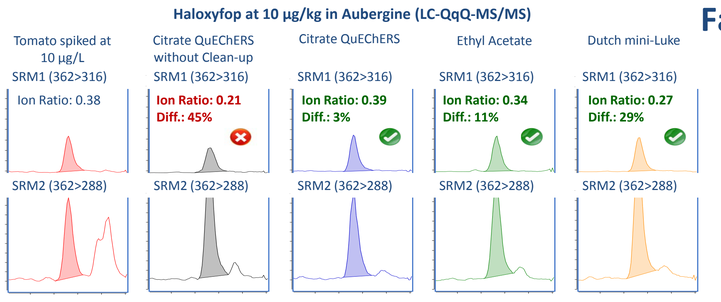
The ten most difficult matrix extracts were selected and spiked at four concentration levels for each extraction method, both for LC and GC. From these analysis, linearity and matrix effect were studied.

Regards linearity, all matrices/extraction methods showed a good response, with residual values lower than 20 % for each concentration level, and correlation coefficient higher than 0.99.

To evaluate matrix effect, slopes obtained for each commodity/extraction method were compared to that one resulting in solvent. In LC, increasing the complexity of the matrix also increase the percentage of compounds showing moderate (20% < x < 50%) or strong (x > 50%) matrix effect, belonging to a suppression phenomenon close to 70 % of results. Contrary to this, in GC a 10 % of compounds as much presented no matrix effect (x < 20%); and, for the rest, close to 98 % of compounds showed enhancement phenomena.

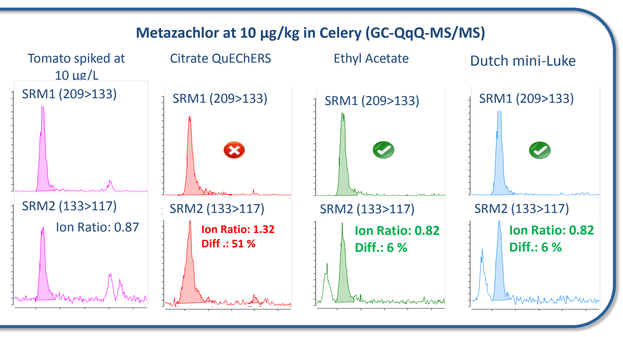


False Negatives Examples



Spiking most problematic matrices, the presence of a interference can lead to a wrong identification, due to the influence on the ion ratio, and providing a false negative result.

As can be seen in the chromatograms, the interferences can also be related to the extraction method.



Conclusions

✓ The percentage of compounds showing a interference in the ± 0.2 min retention time range is similar with all the studied methods. Only differences were observed in avocado where the interferences were higher for ethyl acetate and Dutch mini-Luke extraction methods.

✓ Possible interferences detected in LC are much lesser comparing to GC, because of the 5 times dilution factor. Furthermore this fact can be a consequence of the different ionization source used (EI and ESI).

✓ The presence of interferences can lead to false negatives/positives and difficulties for a good quantification. But the number of these "potential" bad results can be reduced by decreasing the accepted retention time window for each target compound (e.g. ± 0.1 min).

References:
[1] Uclés S., Belmonte N., Mezcua M., Martínez A. B., Martínez-Bueno M. J., Gamón M., Fernández-Alba A. R., *Journal of Environmental Science and Health, Part B, 2014, 49, 557-568.*
[2] Lozano A., de Kok A., Kiedrowska B., Scholten J. and Fernández-Alba A.R., *Food Control, 2015, accepted.*

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