PESTICIDE RESIDUE RESEARCH GROUP

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High increase of analytical sensitivity using microflow liquid chromatography mass spectrometry in pesticide residue determination in fruits and vegetables

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INTRODUCTION

LC high flow rates such as 0.5 – 1 mL/min theoretically can facilitate and increase the signal response of the target compounds when it is coupled to a mass spectrometric detector. But, the flow rate influence on the MS response is very dependent of the interface characteristics and matrix effects. In some ESI designs a decrease to below 0.1 mL/min can be optimum as a consequence of an increase in ion production and sampling. Microsprays with small plumes and low droplet space charge repulsion coupled with adequate ESI source design represents two key aspects in an increasing ion production and sampling. In this work we have explored the capabilities of microflow LC in improving MS signal response and decreasing matrix effects.

Fruits and vegetables are complex matrices as a consequence high matrix effects are likely. To minimize or eliminate the matrix interferences several strategies can be used, as the extracts dilution, which leads to the injection of less matrix into the chromatographic system. Although in this case sensitivity is a key factor, given that it implies also in a reduction of the amount of analytes, the high sensitivity of micro LC-MS systems makes possible the application of very high dilution factors.

QuEChERS extracts of 3 blank matrices (tomato, green pepper and orange) were spiked at five concentration levels with 92 pesticides, diluted 30 times and analysed by microLC-MS/MS. For separation, narrow-bore column was used (0.5 mm x 50 mm x 2.7 µm). Data were used to evaluate matrix effects, limits of detection and quantitation. Reproducibility of peak retention time and peak areas were assessed by 5 no consecutive injections of spiked tomato extract. An application of the method was carried out analyzing 39 fruits and vegetables samples of different kinds and commodity groups (purchased in different supermarkets).

EXPERIMENTAL

Sample handling



Citrate buffered QuEChERS

↓

Blank extracts

↓

Spiking at appropriate level

← Add **IS** (Linuron-d_6) to control the dilution

↓

Diluted **30 times** with AcN:H_2O (10:90)

← Add **IS** (Dimethoate - d_6) to control the injection

↓

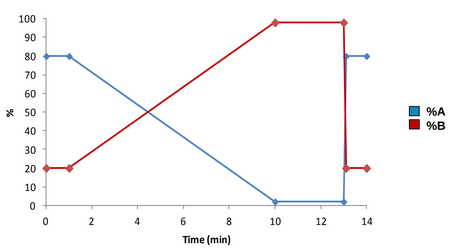
Injection

microLC-MS/MS

System: Ekspert microLC 200 coupled to an 4500 QTRAP ABSciex

HPLC parameters:
- Injection volume: 3µL
- Flow rate: 30µL/min
- Column: HALO C18 2.7 µm 90 Å 0.5x50mm
- Mobile Phases and gradient :

· A → H_2O 0.1% formic acid
· B → AcN 0.1% formic acid



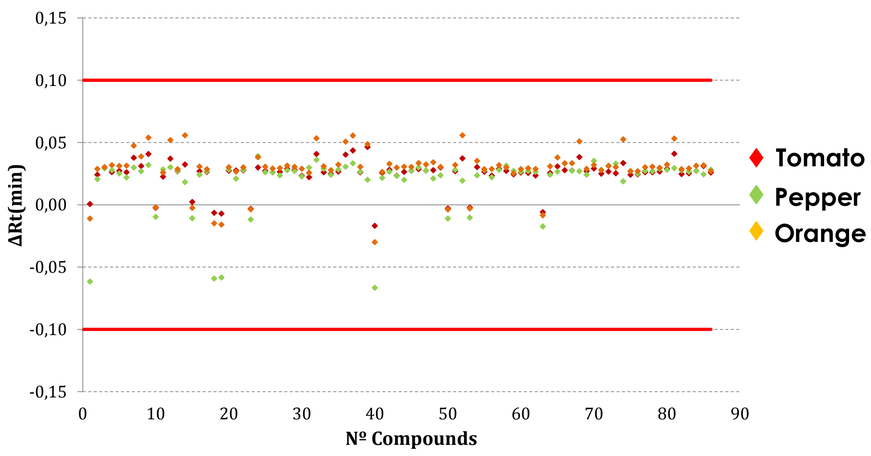
Ekspert microLC 200

MS parameters:
- Ion source: ESI with microFlow electrode
- Polarity: Positive
- Schedule MRM software features

ABSciex 4500 QTRAP

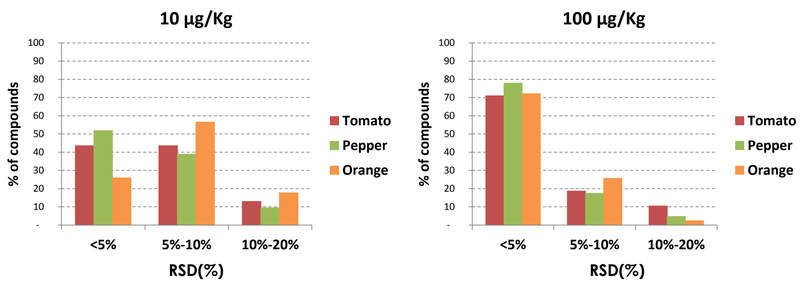
RESULTS

Retention time reproducibility



Difference between consecutive injections of tomato, pepper, orange and standard in solvent spiked at different concentrations diluted 30 times.

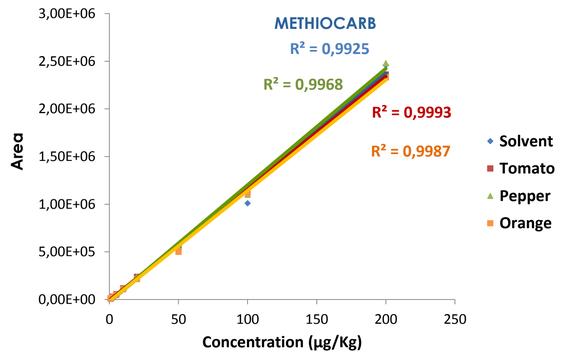
Peak area reproducibility

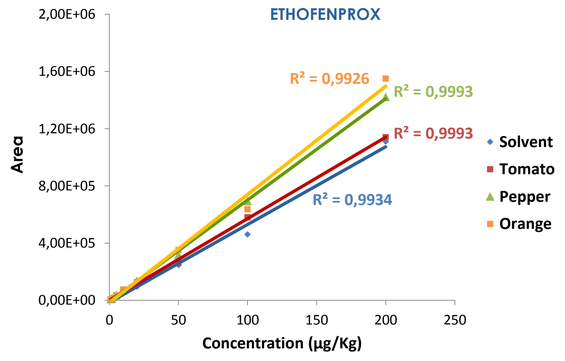


Peak area reproducibility of 5 no consecutive injections of a tomato, pepper and orange spiked at 10 µg/Kg and 100 µg/Kg and diluted 30 times.

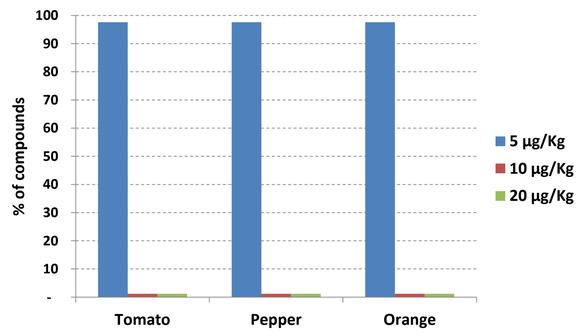
Selected linear response

Matrices diluted 30 times

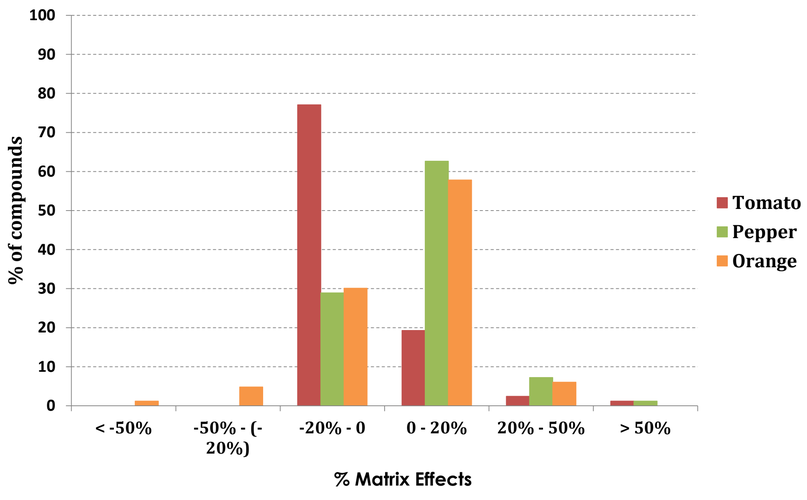




Reporting limits

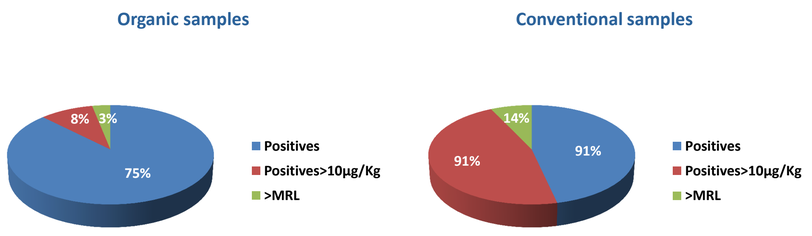


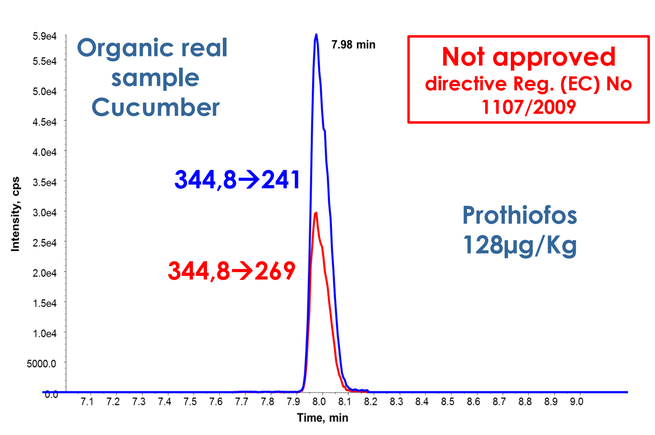
Matrix effects



Matrix effects comparison of the three studied samples (tomato, pepper and orange) diluted 30 times.

Application of the method





The extraction method was applied to 39 fruit and vegetable samples of different kinds and commodity groups (purchased in different supermarkets). 72% were organic and 28% conventional samples. As example, a chromatogram and the concentration of the pesticide found in a organic samples is shown above.

CONCLUSIONS

- The method was validated studying recoveries, reporting limits, linearity , repeatability and matrix effects, giving good results.
- Sensitivity of the system was enough to determine the majority of the pesticides spiked at the lowest level (5 µg/Kg). Only two pesticides presented high reporting limits (fenitrothion 10 µg/Kg and iprodione 20 µg/Kg) in the three studied matrices .
- The commercial microLC pump and narrow bore columns used, assured good retention time as well as peak area reproducibility.
- The percentage positive samples in the organic group and conventional group of samples was 75 and 91, respectively. In total, were detected 35 pesticides, 10 were present in concentrations that exceeded MRL established by European Union and five of them are not approved by regulations in the European Union. Only 3% of the organic samples and 14% of conventional ones had pesticides in concentration above MRL.

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