

Multiresidue method for the quantification of pesticides in fruits, vegetables, cereals and black tea using UPLC-MS/MS

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INTRODUCTION

As the population grows, demand for food consumption and global trade in the food industry has also increased. Hundreds of pesticides are routinely used for crop protection across the globe. The traces of pesticides left in treated products are called "residues". The regulations are in place for Maximum Residue Levels (MRL), that are legally tolerated in or on food or feed when pesticides are applied correctly in accordance with Good Agricultural Practice.

A growing target list of pesticides in complex matrices, and the need for low limits of detection, brings various challenges for multi-residue methods. A multi-residue method for 552 pesticides and relevant metabolites was developed for various food commodities. Extracts from representative commodities of high-water content (spinach), high acid and high-water content (strawberry), high oil and very low water content (soybean), high protein and low water and fat content (wheat flour) and difficult or unique commodities (black tea) were chosen to assess the performance of the UPLC-MS/MS method.

METHODS

Sample Preparation

Samples were immediately homogenized in a food processor and frozen until extraction was performed. The samples were extracted using the QuEChERS methods developed by the EURLs for [fruit and vegetables](#) and [cereals](#) (Figure 1).

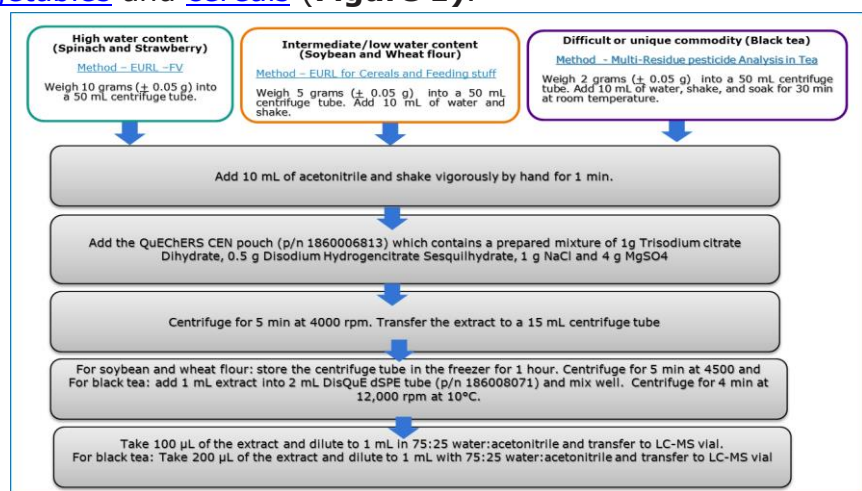


Figure 1. Sample preparation method for strawberry, spinach, soybean, wheat flour and black tea.

Instrumental Conditions

UPLC-MS/MS

UPLC system: ACQUITY UPLC I-Class with FL Sample Manager
Column: ACQUITY UPLC HSS T3 column, 1.8 µm, 2.1×100 mm (186003539)

Post injector mixing kit: 50 µL extension loop (430002012)

Mobile phase A: 5 mM ammonium formate in water + 0.1% formic acid
Mobile phase B: 5 mM ammonium formate in 50:50 MeCN: MeOH + 0.1% formic acid

Injection volume: 5 µL (PLNO mode)

Column temperature: 40 °C

Time	Flow rate	% A	% B	Curve
Initial	0.5	99.0	1.0	Initial
0.5	0.5	99.0	1.0	6
3.50	0.5	60.0	40.0	6
12.50	0.5	15.0	85.0	6
12.60	0.5	1.0	99.0	6
15.00	0.5	1.0	99.0	6

MS Instrument: Xevo TQ-XS

Ionisation: Electrospray

Polarity: positive and negative ion mode

Capillary voltage: +2.0/-2.0 kV

Desolvation temperature: 300 °C

Desolvation gas flow: 1000 L/Hr

Source temperature: 150 °C

Cone gas flow: 150 L/Hr

RESULTS AND DISCUSSION

This multi-residue method was developed for a wide range of pesticides with varying chemistries. A few of these pesticides, such as methamidophos and acephate, are very polar and elute early in the chromatogram. Injecting samples containing moderate organic content (25%) often results in fronting and/or split peaks for the early eluting compounds. Reducing organic content in the sample diluent prior to its injection onto the column may help to improve the peak shape of early eluting analytes. A post injector mixing kit was installed to allow the injection of typical QuEChERS extracts into high aqueous mobile phase without compromising peak shape. This extension loop was placed in between the injector port and column. Before the injection was made, the extension loop is filled with the high aqueous mobile phase, which provides more volume to aid dispersion of the sample into the aqueous solvent prior to transfer onto the column. In this way, a moderate level (25%) of organic solvent can still be used to prepare the sample, in which most of the pesticides remain soluble, whilst still providing good peak shape for the very polar analytes. Figure 2 shows the peak shape of methamidophos with and without the post injector mixing kit; good peak shape was observed with the extension loop fitted, which provided more reliable quantitation and greater sensitivity and hence a lower limit of detection (LOD).

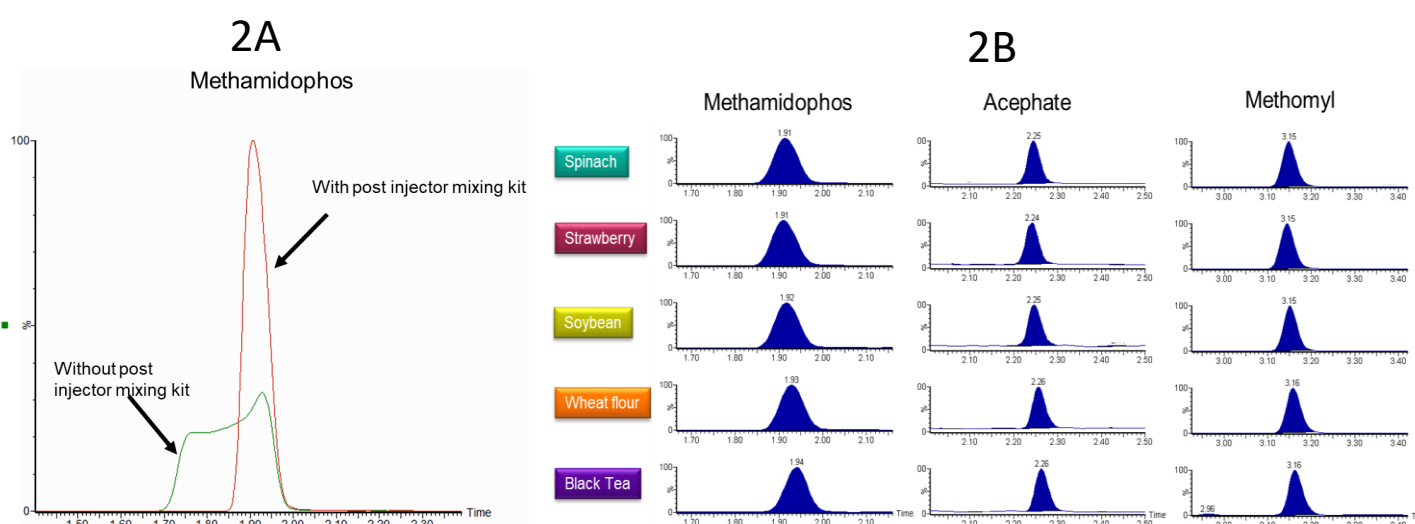


Figure 2A. Chromatogram of methamidophos with (green trace) and without (red trace) the extension loop.

Figure 2B. Chromatograms for some of the very polar analytes across matrices with the extension loop.

Sensitivity, quantification, precision and matrix effects

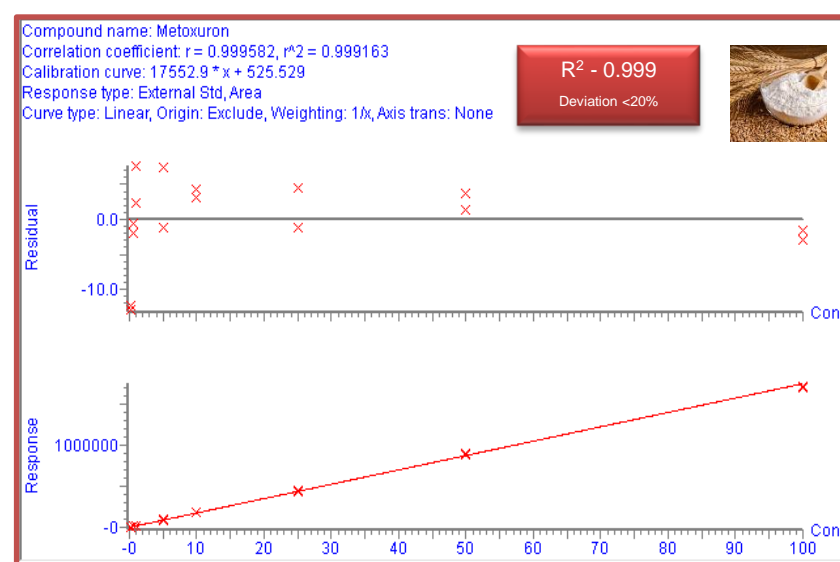


Figure 3A. An example matrix-matched calibration graph for metoxuron in wheat flour

Figure 3B. The percentage of pesticides with RSDs <10% in various matrices at 0.005 mg/kg, 0.01 mg/kg, and 0.05 mg/kg.

Despite the complexity of the various matrices, the LOD (defined here as $S/N > 10$ for both MRMs) for the majority of the 256 compounds tested was 0.005 mg/kg; >90% for spinach, strawberry and soybean, 83% for tea and 79% for wheat flour. The calibration graphs for the majority of the 256 compounds in all the matrices showed values for coefficient of determination (R^2) greater than 0.99 and back-calculated concentrations (residuals) were all within the $\pm 20\%$ tolerance in the [SANTE guidelines](#). Figure 3A shows an example matrix matched calibration graphs for metoxuron, a representative analyte, in wheat flour. The precision of the LC-MS/MS measurements was calculated for the 256 representative pesticides from the replicate ($n=6$) determination of matrix-matched standards at three concentrations (0.005, 0.01 and 0.05 mg kg⁻¹). The precision of the measurement was good with more than 85% of the detected pesticides exhibiting RSDs for peak area of less than 10% (see Figure 3B).

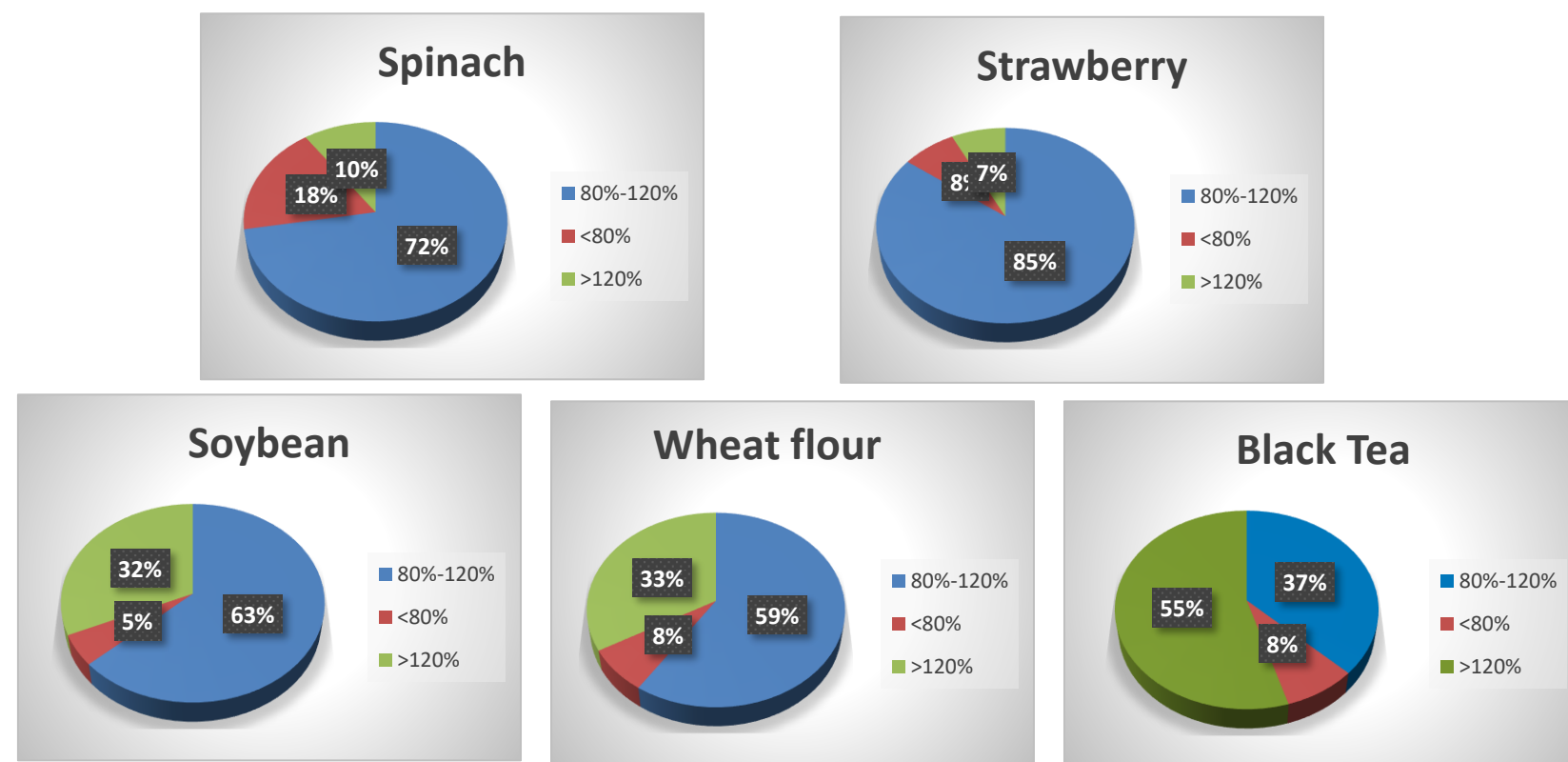


Figure 4. Percentage of pesticides that exhibited significant matrix effects for spinach, strawberry, soybean, wheat flour and black tea.

Matrix effects for the 256 compounds in the various matrices were calculated by comparing the ratio of the slope of the matrix-matched calibration graph for each commodity to that of the solvent calibration graph. Figure 4 summarizes the range of matrix effects observed for each commodity. All commodities show some degree of matrix suppression (response suppressed by >20 %) and enhancement (response of the analyte increased by >20 %) so matrix-matched calibration is recommended. Procedural calibration or standard addition are alternative approaches which compensate for matrix effects and recovery losses.

CONCLUSIONS

- A multi-residue UPLC-MS/MS method has been developed for the determination of 552 pesticides and relevant metabolites for pesticide residue analysis.
- The performance of the UPLC-MS/MS method was evaluated for the determination of 256 representative analytes in extracts from various commodities.
- The post injector mixing kit enables injection of typical QuEChERS extracts into high aqueous mobile phase without compromising peak shape of early eluting analytes.
- Detection of most compounds in matrix-matched calibrants at concentrations below the typical EU default MRL of 0.01 mg/kg was achieved in the five matrices.

