

Euan Ross,¹ JD De Alwis,¹ Defeng Huang,² Jonathon Fox¹ and Eimear McCall¹
¹Waters Corporation, Wilmslow, SK9 4AX, UK. ²Waters Corporation, Beijing, China.

INTRODUCTION

The determination of pesticides in food oils, such as olive oil, has many challenges. Due to the complexity of the matrix, sample clean-up is crucial to ensure a robust methodology, which does not lead to significant contamination of GC liners and columns, resulting in poor chromatography. Traditional approaches to the clean-up of high fat content samples such as olive oil have used a liquid-liquid extraction followed by GPC clean-up. This technique, although still in use today, is no longer attractive due to high solvent consumption and lengthy sample preparation times.

An alternative approach using acetonitrile, either through use of QuEChERS or a solvent extraction, followed by SPE clean-up to remove lipids is becoming increasingly popular in many residue laboratories. In this poster, such an approach is discussed for 150 pesticides in olive oil, with a further evaluation given to the same simple passthrough SPE cleanup for QuEChERS extracts of a variety of fruits, vegetables and cereals.



METHODS

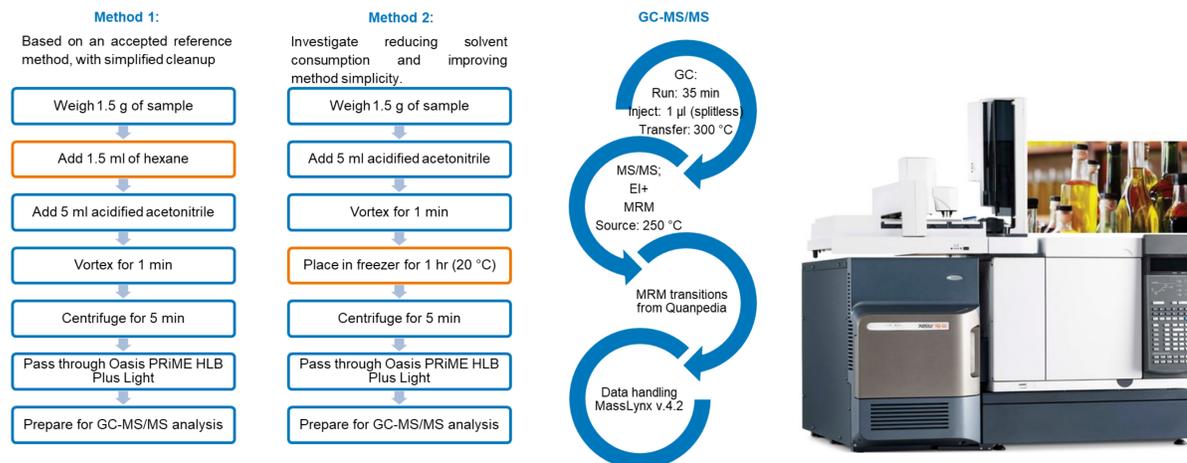


Figure 1. Summarized sample preparation approaches, including extraction and cleanup, and GC-MS/MS methods applied in this study.

Sample preparation, extraction and analysis:

Samples were purchased from retail outlets, bottles inverted and sampled. Spiking all samples with over 150 pesticides, extraction efficiency and simplified sample cleanup was investigated. These methods are summarised in Figure 1 to the left and results are discussed below.

Taking a previously published and accepted reference method for the extraction and cleanup of edible oils, the extraction procedure was replicated and GPC cleanup was replaced by simple SPE pass through, as summarized in Method 1. Method 2 attempted further to reduce the consumption of nonpolar solvents, replaced instead by a freeze out step (as highlighted by orange boxes).

Further to the edible oils analysed as outlined above, a number of fruits, vegetables and cereal samples were also prepared using the traditional QuEChERS extraction, followed by cleanup with Oasis PRiME HLB Plus.

All extracts were acquired on the Xevo TQ-GC (GC-EI-MS/MS) system, with triphenylphosphate added used as internal standard prior to injection. The GC-MS/MS method is briefly summarized. Full method details are available. For more information, scan the QR code below.

RESULTS AND DISCUSSION

ANALYSIS OF EDIBLE OIL SAMPLES: EVALUATING ANALYTE EXTRACTION AND SIMPLIFYING SAMPLE CLEANUP

The extraction optimized in this study yielded improved method recovery for representative pesticides, when compared to the traditional hexane and acetonitrile extraction procedures. The impact of the pass through cleanup step was also evaluated separately, where analyte recoveries were >70% for all analytes, showing negligible loss of the analytes during cleanup.

While hexane is a useful solvent for the extraction of nonpolar contaminants (such as fat), one observation when using the approach is poor recoveries of some pesticides, specifically organochlorines, due to their solubility. This is shown in Table 1, where the recoveries for Method 1 are significantly less than the 70% limit indicated in the SANTE guidelines.

By avoiding the use of hexane, and instead applying a defatting step by freeze out, significant improvements were observed for the recoveries of many of the representative organochlorine compounds. Some low recoveries (< 60 %) were still observed for a number of analytes, suggesting limited extraction using this generic acetonitrile procedure from these complex food types.

Method recoveries for cleanup by Oasis PRiME HLB passthrough were also evaluated (by spiking samples post extraction). These results, as shown in Table 1, yielded recoveries > 70%, showing that the spiked analytes were not retained on the SPE cartridge during either Method 1 or 2.

While the overall method recovery demonstrated improved performance over traditional techniques, additional investigations were conducted to improve the linearity and repeatability. An alternative type of calibration, namely procedural standards, was employed. This mode of calibration compensates for low extraction efficiency and matrix effects and showed excellent improvements in terms of accuracy and repeatability, where the method's trueness (for edible oil samples spiked prior to extraction and cleanup) ranged from 99.2 to 108.5 % for a selection of challenging organochlorine pesticides. Excellent linearity, over the calibration range of 0.005 to 0.1 mg/kg, was achieved for all pesticides with coefficients of determination > 0.995 with residuals <20%. The method's accuracy, repeatability and bias was evaluated from the analysis of spikes at 0.01 mg/kg (n=5), 0.02 mg/kg (n=5) and 0.1 mg/kg (n=5).

Following in-house validation of this optimized extraction, cleanup and GC-EI-MS/MS analysis, the method is shown to be simple, quick, solvent friendly, reliable and fit for the routine determination of multi-residue pesticides in edible oils. Taking a set of representative compounds these results are discussed in terms of analyte extractability, cleanup from matrix and approaches to improve quantitation.

Table 1. Recoveries are shown for a selection of more challenging analytes, where the hexane based defatting step shows low recoveries while replacing this solvent based extraction of nonpolar with a freezing step, analyte recovery is improved. The final column (Oasis PRiME HLB Step) demonstrates that minimal analytes are retained on the Oasis PRiME HLB device, following simple passthrough.

Compound	Method 1 % recovery	Method 2 % recovery	Oasis PRiME HLB Step % recovery
Aldrin	29.6	58.7	93.5
Alpha-BHC	53.0	98.7	96.5
Gamma-BHC	71.2	101.0	98.0
Chlordane, cis-	63.5	67.7	97.0
DDT, o,p'-	45.3	52.7	93.0
Dieldrin	53.0	46.7	74.5
Endrin aldehyde	71.5	100.7	91.0
Heptachlor	41.3	79.0	94.5
Hexachlorobenzene	15.2	38.0	77.5
Nonachlor, trans	37.6	60.7	84.5
Mirex	24.8	27.7	86.0
Pentachloroanisole	26.4	57.7	86.0

ANALYSIS OF CUCUMBER, GRAPE AND RICE SAMPLES: APPLYING SIMPLIFIED SAMPLE CLEANUP TO QUENCHERS EXTRACTS

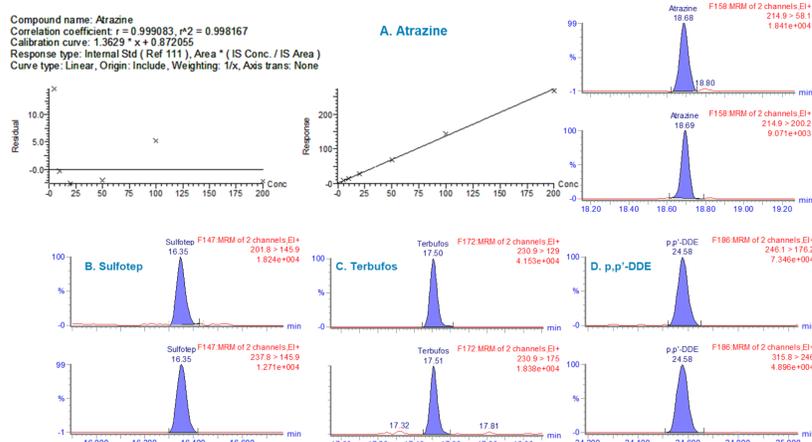


Figure 2. A Example of the matrix matched calibration curves and lowest spiked QC (0.01 mg/kg) shown for atrazine in rice. Further examples of analytes at the targeted LOQ (0.01 mg/kg) is shown for B. sulfotep, C. terbufos and D. p,p'-DDE, all spiked in rice, prior to extraction with QuEChERS and cleanup using Oasis PRiME HLB Plus devices.

The traditional QuEChERS extraction was applied to a selection of high aqueous and high starch with low water and fat content commodities and the same cleanup procedure of passthrough SPE was evaluated. The method's performance was assessed for trueness, reproducibility, quantification and identification of 208 pesticides and associated metabolites in cucumber, grape and rice. For each commodity (n=3), matrix matched calibration curves were generated (Figure 2A) and replicate spikes (n=6) were extracted at three concentrations (LOQ, 2x LOQ and 5x LOQ). The measured percentage recovery and repeatability for a representative selection of 15 pesticides across the commodities tested is shown in Figure 3. Further details on recovery and repeatability for all 208 pesticides at the required LOQ across each commodity are available by scanning the QR code below.

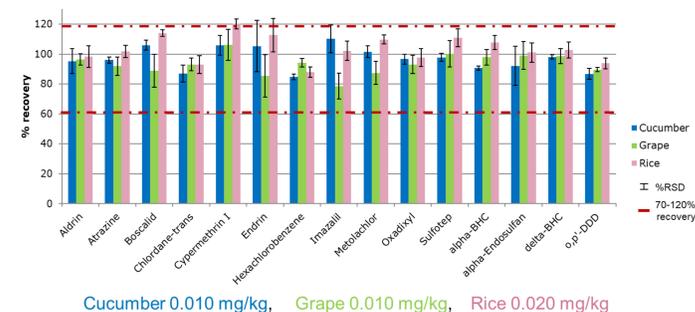


Figure 3. The measured recoveries (trueness, between 70 and 120%) and repeatability (%RSD, < 20) of pesticides spiked at the required LOQ



Figure 4. QuEChERS extract of cucumber (green extract), passed through Oasis PRiME HLB (clearer yellow extract), where chlorophyll has adhered to the PRiME HLB device.

CONCLUSIONS

- This work looked at replacing traditional cleanup techniques, with cleaner, simpler and quicker technologies, by looking at analyte recovery through the sample preparation steps.
- For edible oils, by replacing the hexane defatting and GPC cleanup with a simple defatting step by freezing out and passthrough SPE cleanup, respectively, a greener, less solvent intensive method is achieved for these highly complex samples. All 150 pesticide analytes yielded satisfactory recoveries when applying a passthrough SPE cleanup using Oasis PRiME HLB Plus, while some analytes still showed low recoveries from the samples.
- For the samples of fruit, vegetables and grains analytes were extracted using the renowned QuEChERS protocol, while the traditional dSPE cleanup required for extracts prior to GC-MS/MS analysis was replaced with the above mentioned passthrough SPE cleanup using Oasis PRiME HLB Plus. More than 95% of the pesticides showed measured recoveries within the range of 70% to 120% range and repeatability (RSD) was <20% (n=5) for all compounds in all commodities.

REFERENCES

1. European Union (2019), Document No. SANTE 12682/2019. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed.

Scan the QR bar code for more information

