MAXIMIZING THE METHOD'S PERFORMANCE AT THE ANALYTICAL SYSTEM'S FIRST PORT: SAMPLE INJECTION

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Dimple Shah,[1] Euan Ross, [2] Simon Hird, [2] Stuart Adams [2] and Eimear McCall [2]
1 Waters Corporation, Milford, MA, USA; 2 Waters Corporation, Wilmslow, United Kingdom.

INTRODUCTION

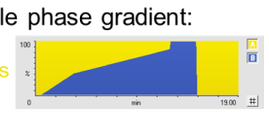
The generic extraction protocols employed for the determination of multiple pesticides, for example QuEChERS, typically results in a highly organic extract, which can impact peak shape, detection levels and repeatability in reverse phased LC-MS/MS analyses. Typically, solvent exchange and/ or dilution with water improves compatibility of the extract with the LC starting gradient, often adding additional time, cost and error to the analysis.

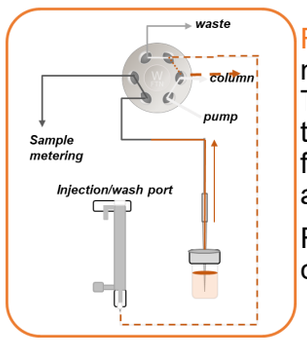
In this poster, we investigate a number of injection modes available on ACQUITY chromatographic sample managers and their applicability in the analysis of pesticide residues and metabolites in foodstuffs. The benefits in the improved detection and quantitation of analytes is reported by considering optimised parameters and functionality to automate additional steps in the analytical workflow.

METHODS

A number of foodstuffs were prepared using QuEChERS CEN extraction method. The resultant, crude extract in 100% acetonitrile were analysed on LC-MS/MS, using fixed loop (FL) and/or flow through needle (FTN) injector systems. A typical reverse phase method was employed throughout, running an ACQUITY BEH C_{18} column with ammonium formate/formic acid in water and acetonitrile. The mobile phase gradient is summarised:

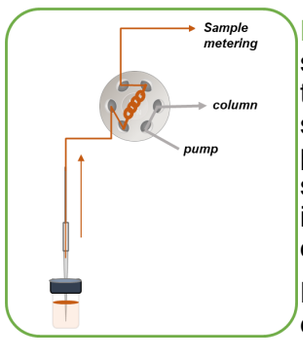
Mobile phase gradient:
Starting at
99% aqueous
1% organic





Flow through needle (FTN) mechanism of sample aspiration. Thereafter, the needle moves to the injection/ wash port and the flow is directed to the column, with a pre-injection wash option.

For more information, scan the QR code below.



Fixed loop (FL) mechanism of sample aspiration and injection onto the analytical column is summarised. The sample's flow path through the automated switching port will depend on the injection mode applied (full, partial or partial with overfill).

For more information, scan the QR code below.

RESULTS AND DISCUSSION

1. Standard Loop Injection

In recent years, many analytical methods have moved from traditional high dispersion LCs (HPLC) to more modern LCs (U(H)PLC) operating at higher pressures. While significant gains are achieved in terms of increased peak capacity, reduced run times and solvent consumption, these lower dispersion systems can be less forgiving to mis-match of the mobile phases' and extract's solvents, resulting in obvious fronting and/ or splitting of early eluting analytes.

When the QuEChERS extract is evaporated and reconstituted or diluted to a ratio more compatible with the starting gradient conditions, the use of standard FTN injection or PLNO (partial loop with needle overfill) on FL are appropriate for a low injection volume of circa 1 µl, which is common in many multi-residue methods today. While this dilution is required for repeatable peak shape and reliable quantitation, some drawbacks are summarised in **Table 1**. Automated solutions, within the sample manager, are considered below.

Table 1. Challenges introduced by extract dilution

	Drawback	Impact
1.	Additional manual steps	Time consuming and increases risk of random and human error
2.	Stability of all analytes in diluted extract	Analytes may crash out of the aqueous extract during the batch analysis
3.	May require higher injection volume to meet the detection limit	May result in additional peak splitting and/ or asymmetry

2. Diluting Extract at Injection

While the dilution of QuEChERS extracts are commonly carried out routinely by analysts, these steps can be time consuming and introduce additional sources of error. Getting the ratio of QuEChERS extract to water correct is important, as diluted extracts in storage during an extended analytical run may result in analyte loss due to precipitation, insolubility in the aqueous solvent, binding to the solvent/ vial during their time in the sample manager. The risk of increased error and loss of analytes in diluted extracts may be overcome by diluting the neat QuEChERS extract, automatically, just prior to injection. This is shown in **Figure 2**, where the peak shape of methamidophos, the first eluting analyte shows significant peak splitting (A). Using Auto Addition in MassLynx (B), aliquots were taken from different vials (two, in this example) and injected as a single injection onto the analytical system (C).

This functionality has reduced the tedious steps of pipetting, capping and mixing the sample extracts in manual dilution. Furthermore, the error, including human and random, were reduced by employing the Auto Addition functionality with the sample manager. Repeatability (%RSD) of replicate QC samples was improved when compared against manual dilution by pipette.

More information and examples on the use of Auto Addition is available.[1]

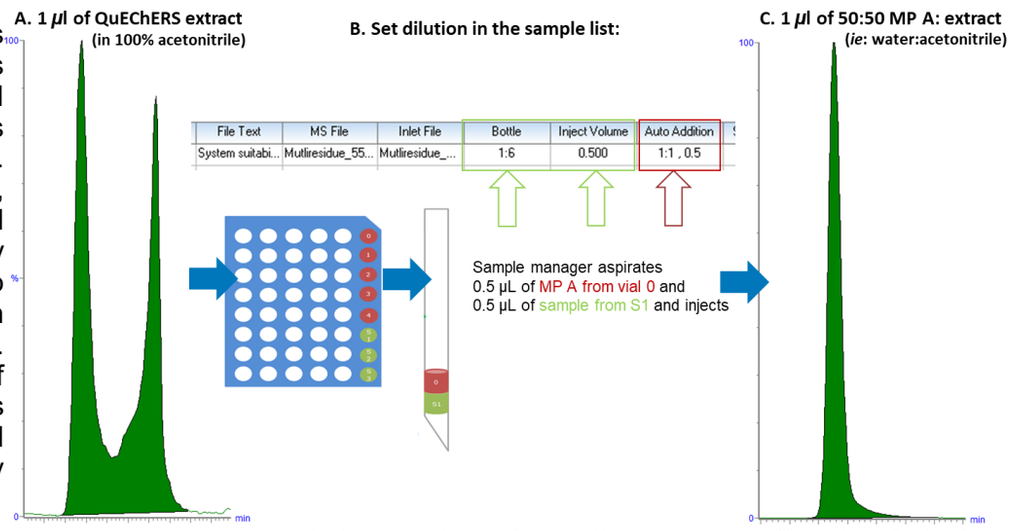


Figure 1. Improvement of peak shape for methamidophos (C) using Auto Addition in MassLynx (B) to inject and automatically dilute. This provided improved peak shape and sensitivity relative to splitting peaks observed (A) when injecting 1 µl of the neat QuEChERS extract in 100% acetonitrile.

3. Focusing Larger Volume Injections

Although 75:25 water:acetonitrile extract is considered a good compromise in multiresidue analysis of pesticides (allowing acceptable solubility, stability and peak shape), injecting larger volumes into a 95 or 99% aqueous starting gradient can result in additional peak fronting and/ or splitting. Again taking methamidophos as an example, **Figure 2** shows the resulting peak splitting due to 5 µl of the extract in 75:25 solvent. By extending the path to the column (a 50 µl extension loop was inserted between the column port and the analytical column), the extract and mobile phase experience better mixing, allowing improved analyte, and thus peak focusing at the start of the run. In this work, similar improvements in peak shape were observed for acephate, cyromazine and methomyl, while no significant negative effects were observed for all other ~550 pesticides. More information and results from this work are available. [2]

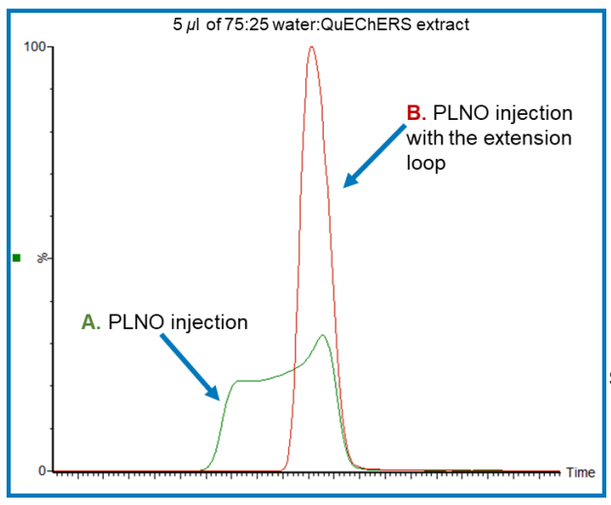


Figure 2. Injecting a larger volume of sample into the highly aqueous gradient results in peak splitting of the first eluting analytes. By adding a post injector mixing kit, the increased mixing volume improves solvent compatibility. This provides Gaussian peak shape, which improves the sensitivity, repeatability and data review/ processing speed, without impacting other analytes in the run.

CONCLUSION

- **The generic extraction of multiple residues by QuEChERS procedure has revolutionised efficiency in pesticides analysis. Depending on the sample manager and injection mode used, much functionality is available to help streamline analytical workflows, improve efficiency and method reliability.**
- **Use of Auto Addition on FTN sample managers allows for the programable dilution from the sample tray, allowing for reduction in tedious manual steps and analyte loss while improving repeatability and peak integration efficiency during processing.**
- **When applying additional dilution to minimise matrix effects or when running lower sensitivity detectors, larger injection volumes (*e.g.* 5 or 20 µl) may be required to achieve the detection limits. Incorporating the extension loop onto the FTN or FL system allows for such injection volumes to be employed, without compromising the peak shape or method reliability.**

Scan the QR bar code for more information

REFERENCES 1. Waters' Application Note 720005559en: Available here 2. Waters' Poster PV-22 at EPRW 2020: Available here

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