



## A Novel Mass Screening Solution Incorporating the UNIFI Scientific Information System for the Routine Analysis of Pesticide Residues at Regulatory Limits in Food

---

Dominic Roberts, Michael McCullagh, Sara Stead, Gareth E. Cleland, Dimple D. Shah, Lauren Mullin, Jennifer A. Burgess

Waters Corporation



---

## Abstract

In this application note, the performance of the new pesticide screening solution was assessed via the analysis an independent proficiency test sample.

The Pesticide Screening Application Solution with UNIFI has been designed to confidently report the presence and absence of pesticide residues at Maximum Residue Limits (MRLs).The solution streamlines data analysis with customizable workflows leading to the rapid analysis of complex matrices using a scientific library functionality with flexible reporting templates.

## Benefits

The Pesticide Screening Application Solution with UNIFI has been designed to confidently report the presence and absence of pesticide residues at Maximum Residue Limits (MRLs). The solution streamlines data analysis with customizable workflows leading to the rapid analysis of complex matrices using a scientific library functionality with flexible reporting templates.

---

## Introduction

Current trends indicate that in excess of 500 pesticides are routinely used on a global basis. In addition, countries throughout the world have different regulations concerning licensing and MRLs. With increasing global trade, there is a requirement for multi-analyte screening strategies capable of efficiently detecting residue violations in the interest of consumer safety. Recent advances in MS and separations technology have demonstrated a strong potential to improve the analysis of complex samples that often result from the extraction of food samples. For example, these include advances in MS sensitivity and increased peak capacity where UPLC technologies are deployed. This study shows the performance of a new benchtop QToF-based platform incorporating StepWave and QuanTof technologies combined with UNIFI. UNIFI is a revolutionary scientific information system specifically designed for non-targeted accurate mass screening applications that enables scientists to test and report more samples for an increasing number of residues and contaminants. UNIFI captures high resolution, accurate mass LC-MS data, and performs rapid processing for confirmation and quantification of residues in a single software environment.

The benefits of full spectra acquisition with the specificity provided by accurate mass measurement are well understood, and include the ability to perform historical data review as well as non-targeted screening experiments. StepWave technology provides enhanced sensitivity with excellent linear dynamic range, as required for routine quantitative pesticide screening. Such enhancements in ToF hardware technology have improved the precision and accuracy of generated data, facilitating the creation and utilization of more intelligent data processing software packages.

In this application note, the performance of the new pesticide screening solution was assessed via the analysis of an independent proficiency test sample. Comparison between median calculated concentrations and Z-Scores reported for the sample were used to determine the performance of the new approach to pesticide screening.

---

## Experimental

### LC conditions

System:

ACQUITY UPLC I-Class

Run time:	15 min
Column:	ACQUITY UPLC BEH C <sub>18</sub> 2.1 x 100 mm, 1.7 μm
Column temp.:	45 °C
Mobile phase A:	10 mM ammonium acetate dissolved in water
Mobile phase B:	10 mM ammonium acetate dissolved in methanol
Flow rate:	0.45 mL/min
Injection volume:	5 μL

## Gradient

	Time (min)	Flow rate (mL/min)	%A	%B
1	Initial	0.45	98	2
2	0.25	0.45	98	2
3	12.25	0.45	1	99
4	13	0.45	1	99
5	13.1	0.45	98	2
6	15	0.45	98	2

## MS<sub>E</sub> conditions

Mass Spectrometer:	Xevo G2-S QTof
Ionization mode:	ESI+
Capillary voltage:	1 kV
Sample cone:	20 V
Source temp.:	120 °C
Desolvation temp.:	550 °C
Desolvation gas:	1000 L/H
Reference mass:	Leucine enkephalin [M+H] <sup>+</sup> = 556.2766
Acquisition range:	<i>m/z</i> 50 to 1200
Acquisition rate:	4 spectra/s
Collision energy low energy:	4 eV
Collision energy ramp high energy:	10 to 45 eV

## Sample preparation and analytical conditions

Sample extracts, matrix matched calibrants, and quality control samples generated for an EU-RL proficiency test (FV-13 2011) were generously supplied by WIVISP, Brussels, Belgium. A mixed pesticide standard containing 135 pesticides was prepared at six different concentrations. Bulk blank extract was prepared according to a standard liquid-liquid extraction protocol for mandarin. Following the solvent extraction step, the extract was aliquoted and spiked using 50 µL of the appropriate spiking solution to give concentrations equivalent to 1, 5, 10, 20, 50, and 100 µg kg<sup>-1</sup> bracketing the EU “blanket” MRL of 10 µg kg<sup>-1</sup> for pesticide residues. The spiked solvent extracts were diluted 10-fold in water, prior to analysis. The resulting final solution concentrations were 0.1, 0.5, 1, 2, 5, and 10 ng mL<sup>-1</sup>.

## Results and Discussion

Within UNIFI, pesticides are screened using the scientific library, which allows identification based on the accurate mass of the parent, including common adducts like  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4$ , retention time, the presence of fragment ions, as well as isotopic patterns and intensities. The ability to identify pesticides using multiple compound criteria enables the analyst to be confident in making an identification, while speeding up the review process by allowing user-defined filters and workflow steps to be applied. The use of workflow steps in conjunction with user-defined filters provides an efficient approach to review both qualitative and quantitative analysis in a single software environment. Figure 1 shows an example view that can be configured by the user. In this case, the extracted ion chromatogram of the selected pesticide from the current injection is displayed along with the low energy (precursor) and high energy (fragment) spectra.

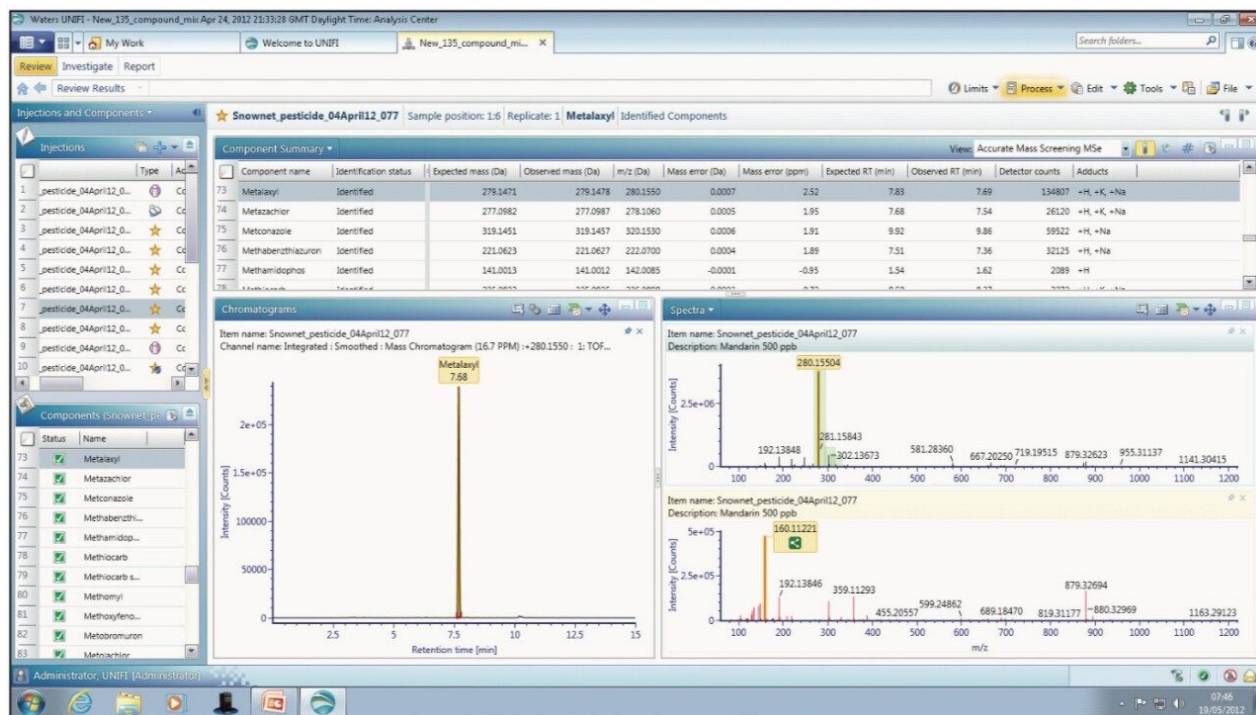


Figure 1. UNIFI component summary table (upper data panel) shows the components identified in the mandarin sample.  $\text{MS}^E$  data simultaneously acquired precursor and fragmentation spectra for metaxyl (lower data panel).

In addition, a summary table, which is also user-configurable, is included to show further information (such as mass error, adducts identified, retention time, and isotope match) to assist with confirmation of the

analyte identity. The pesticides identified in a sample can also be quickly viewed in the component plot along with their expected masses and structures, as shown in Figure 2. Another useful feature of UNIFI is the summary plot, which is shown in the example view in Figure 3. This plot allows the user to view the concentration of pesticides across the sample set, enabling rapid detection of any pesticides present in blanks or samples, and determining if the concentration varies between the injections.

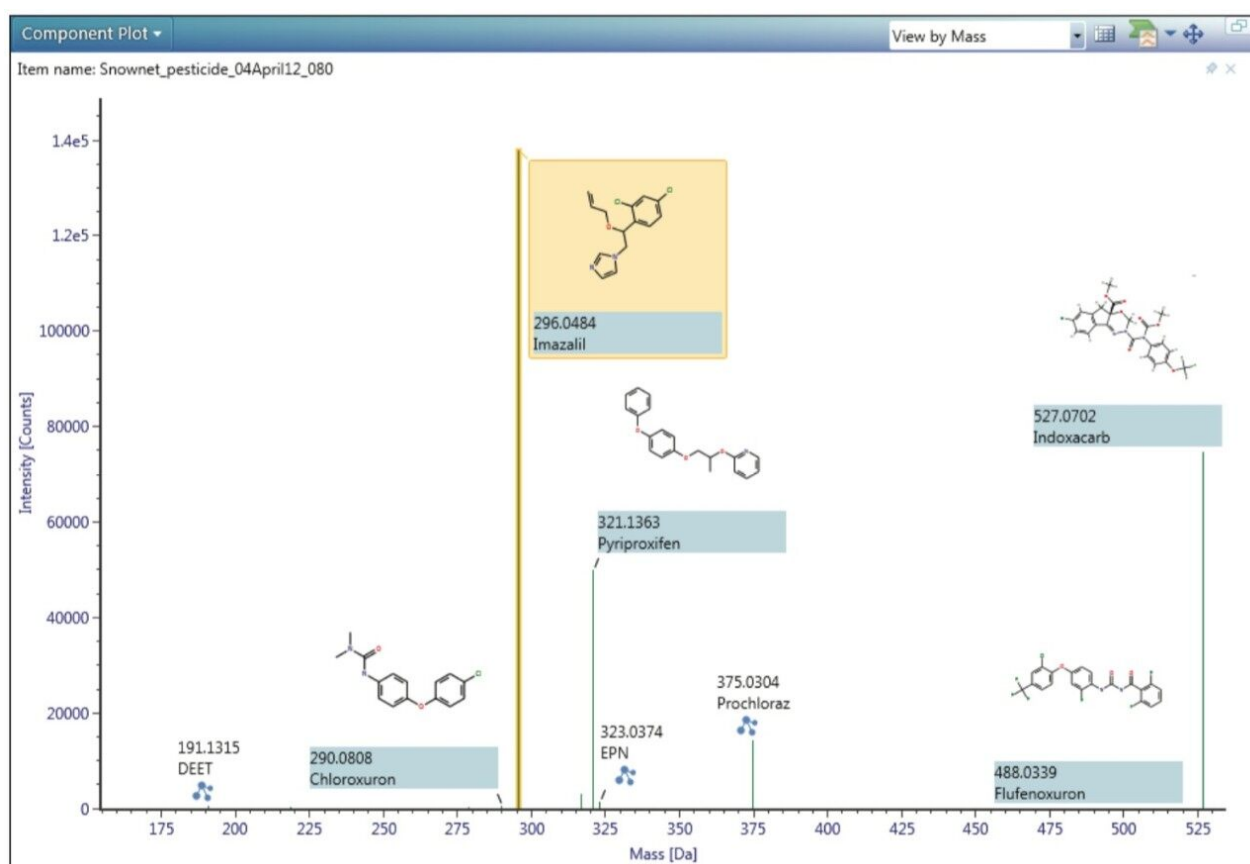


Figure 2. UNIFI component plot view shows a summary of the pesticides identified in the sample (FV-13 2011) and their relative intensities. The peaks are annotated with the expected accurate mass and structures.



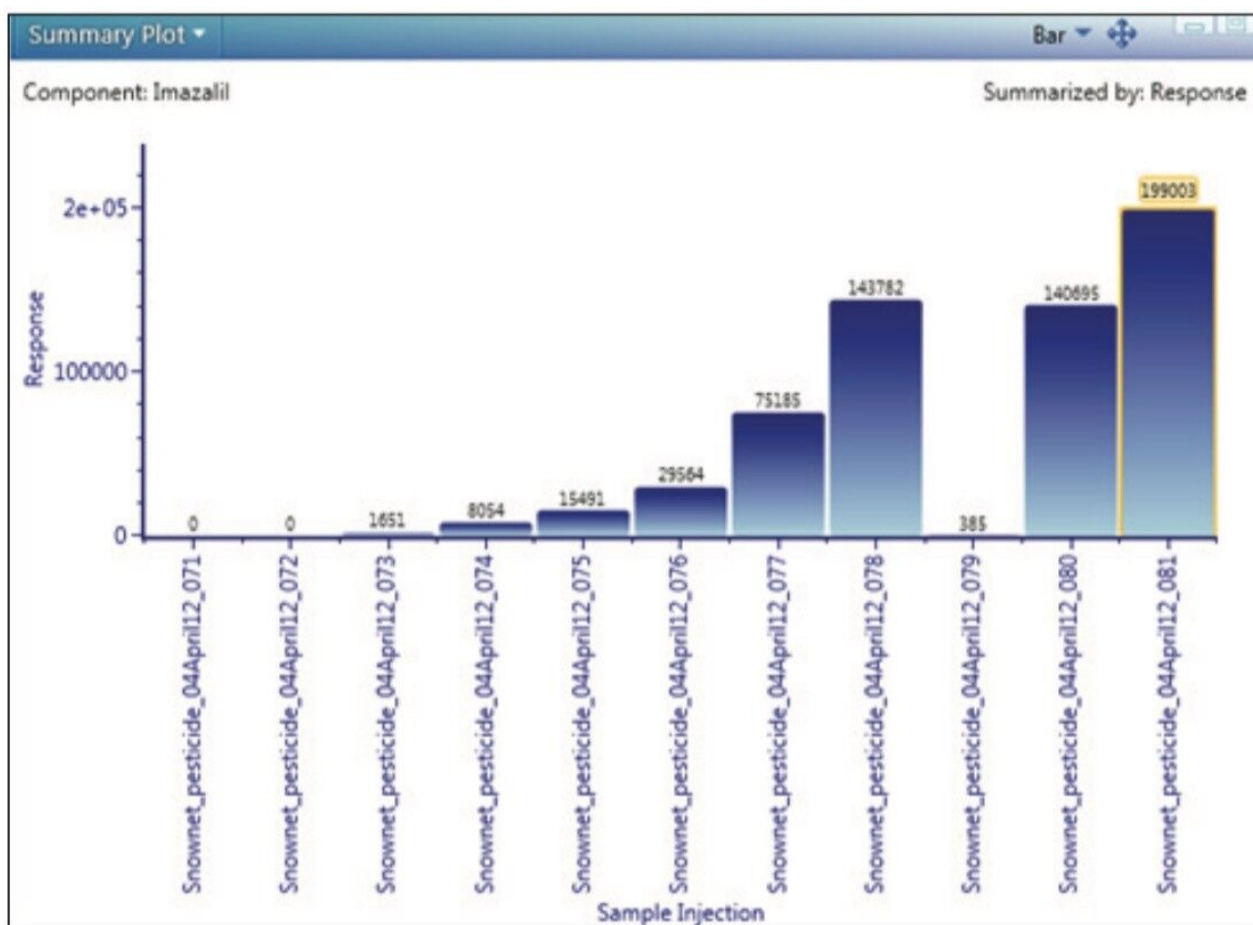


Figure 3. UNIFI summary plot view showing the response across the calibration standards and in the proficiency test sample for imazalil. This summary plot can be customized to show any parameter important to the user, for example, mass difference across the batch or calculated concentration of the analyte.

The user-defined filters enable selection of matches that meet specific criteria. In this way, a user can choose how to review the data in a step-wise fashion. Those pesticides that are identified with high confidence (as defined by the user) can be reviewed rapidly. If there are matches that fail defined criteria, they can be filtered out to be reviewed with additional information. The criterion that has failed the acceptable limit can be flagged to be easily recognizable. Example criteria that could be flagged include an accurate mass shift or questionable isotope ratios. In addition to screening for the presence or absence of pesticides, quantification is also configurable within the same view. This allows the analyst to filter those pesticides that are above an action concentration (for example, 0.5 MRL), thereby improving laboratory efficiency by ensuring that noncompliant samples are accurately detected and reported.

The mandarin extract was screened for the presence of pesticide residues using a target list of LC amenable



pesticides. For each of the pesticide residues identified, calculated concentrations were determined using external standard calibration. The results were compared to the published median values obtained from approximately 150 laboratories who participated in the EU-RL proficiency test (FV-13 2011). All of the eight LC amenable analytes present in the sample were correctly identified and quantified using the new pesticide screening solution, as shown in Table 1. In addition, two GC amenable compounds were also detected at the concentrations present in the sample. The Z-Score values for these analytes were also calculated (according to the same formula used by the proficiency test organizers). Nine out of the 10 analytes detected had Z-Score values less than 2.0 and were classified as "Acceptable." For one of the analytes, EPN, most commonly analyzed using GC, the Z-Score value was 2.7 and classified as "Questionable." For all 10 analytes detected, the mass accuracy (for both precursor and fragment ions) was less than 3 ppm (exceeding the SANCO 12495 requirement of 5 ppm). The quantitative performance of the instrument, in the presence of a complex matrix, was excellent, as demonstrated by the correlation coefficient values.

Marker residue definition	Analyte	Assigned value (mg/kg)	Found value (mg/kg)	Z-Score	Z-Score classification	Correlation coefficient (R <sup>2</sup> )	Mass Accuracy (ppm)
Sum of benomyl and carbendazim expressed as carbendazim)	Carbendazim	1.25	0.97	-0.9	Acceptable	0.989	1.49
EPN	EPN*	0.42	0.70	2.7	Questionable	0.985	-1.75
Imazalil	Imazalil	1.30	1.36	0.2	Acceptable	0.999	0.73
Sum of the isomers S and R	Indoxacarb	0.79	0.94	0.7	Acceptable	0.993	-0.32
Sum of methomyl and thiodicarb expressed as methomyl	Methomyl	0.20	0.10	-1.9	Acceptable	0.998	-0.95
Oxamyl	Oxamyl	0.13	0.14	0.3	Acceptable	0.992	1.69
Only parent compound	Prochloraz	0.30	0.43	1.8	Acceptable	0.984	-0.93
Pyriproxyfen	Pyriproxyfen*	0.44	0.50	0.6	Acceptable	0.994	0.30
Sum of spinosad A and spinosad D expressed as spinosad	Spinosad	0.61	0.89	1.8	Acceptable	0.998	-0.68
Thiabendazole	Thiabendazole	0.81	0.85	0.2	Acceptable	0.990	2.27

*Table 1. The comparative performance of the pesticide screening solution for the LC amenable pesticides identified in an EU-RL proficiency test sample (mandarin FV-13 2011) assessed against the published median calculated results. \*Compounds normally analyzed using GC.*

Analyte specific information that can be used for confirmation purposes, such as elemental composition and fragmentation pathway, can be routinely generated from the accurate mass spectral data acquired within a single injection. Processing simultaneously acquired accurate mass precursor and fragment ion data through

the new scientific information system has enabled the pesticide residues to be confirmed with confidence and very low false positive rates.

The calibration series generated for imazalil is shown in Figure 4. Correlation coefficients of the order of  $\geq R^2 = 0.99$  and residuals of  $<5\%$  were routinely obtained in matrix extract. The component summary, component details, and component plots are also illustrated, providing easily accessible information to further enhance confidence in the results and accelerate the review process.

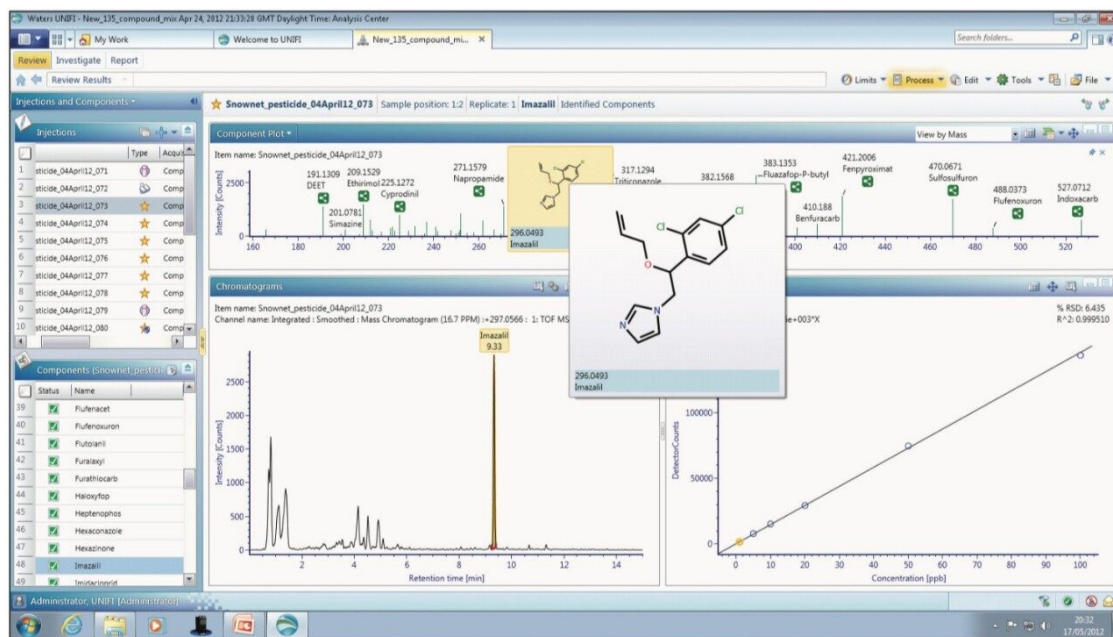


Figure 4. UNIFI component plot (upper data panel) shows the pesticide residues identified in a test sample and the calibration series for imazalil (1 to 100 ppb) and correlation coefficient  $R^2 = 0.999$ .

## Conclusion

Enhancements in both hardware and software technology have contributed to the development of a robust ToF-based solution for pesticide residue screening. These have contributed to the development of the Waters Pesticide Screening Application Solution that allows the routine detection, identification, and quantification of pesticides in complex matrices at MRL concentrations. In this study, residuals of  $<5\%$  and mass accuracy in the order of 2 ppm were obtained over the dynamic range of the study. A good correlation to EU-RL

proficiency test sample (FV-13 2011) was seen for the LC amenable pesticides, as demonstrated by the Z-Score values ( $\leq 2$ ). Structural elucidation information for all of the identified pesticides was generated by collecting simultaneous acquisition of accurate mass precursor and accurate mass fragment ions. Processing simultaneously acquired accurate mass precursor and fragment ion data through a new scientific information system enabled pesticide residues to be confirmed with confidence, minimized false positive rates, and accelerated the review process to ultimately increase laboratory throughput.

---

## Featured Products

[ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>](https://www.waters.com/134613317)

[Pesticide Screening Application Solution with UNIFI <https://www.waters.com/134682906>](https://www.waters.com/134682906)

720004491, November 2012