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UHPLC Analysis of a Pesticide Formulation Using the ACQUITY Arc System with PDA, Mass Detection, and Empower 3 Software

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APPLICATION BENEFITS

- Enhanced confidence in the profiling of impurities using PDA and mass detection.
- Structural similarities between the active ingredient and unknown components can be identified in a single analysis.
- Ease of use with single point control data analysis and reporting via Empower® 3 Software.
- Dual-flow paths that emulate HPLC and UHPLC separations aid method development and transfer.

WATERS SOLUTIONS

ACQUITY® Arc™ System

2998 Photodiode Array (PDA) Detector

ACQUITY QDa® Detector

CORTECS® C_{18}+ Column

Empower 3 Chromatography Data Software

KEY WORDS

Pesticide, formulations, mass detection, UHPLC, impurity identification, tebuconazole, triazole fungicides, insecticide

INTRODUCTION

Crop protection products provide solutions that decrease crop damage resulting in a food supply that is plentiful and of high quality.[1] In the agricultural chemicals industry the analytical quality control of pesticide products is very important to ensure that a consistent and effective product reaches the customer.[2] Detection, characterization, and quantitation of the active ingredient/s and all other components in the formulation including impurities and degradation products are necessary to support product development, quality control, and product registration. Liquid chromatography (LC) techniques with photo diode array (PDA) detection have been used for the routine analysis of formulation samples.[2-4] The addition of a mass detector in conjunction with UV detection can increase the specificity and selectivity of methods used during analytical testing to provide additional information about a sample in a single analysis.

In this application note, we present the analysis of a commercially available pesticide formulation which contained two active ingredients (AI): an insecticide AI 1, and tebuconazole, a triazole fungicide, AI 2 (Figure 1). The triazole fungicides are a commonly used group of pesticides due to their potent activity against a broad spectrum of crop diseases.[5] The analysis of the formulation employed UV and mass detection and a dual-flow path liquid chromatography system capable of emulating HPLC or UHPLC separations.[6] The ACQUITY Arc System enables existing HPLC methods to be performed, while also allowing the choice of transitioning to a UHPLC method that employs sub-3 µm particles for higher efficiency chromatographic separations.

Empower 3 Software was used for data acquisition and analysis, generating results which were used to flag impurities greater than specified %Area levels. Empower's Custom Fields allowed extra information to be derived from the results as custom calculations which were reported using tailored methods. The combined detection capabilities and data analysis provided the initial structural characterization of the unknown components.

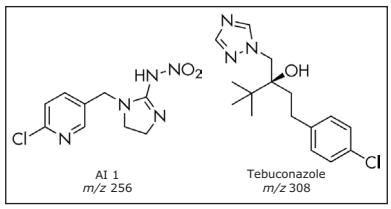


Figure 1. Structures and m/z for AI 1 and tebuconazole.

EXPERIMENTAL

Instrumentation and software

All separations were performed on a Waters® ACQUITY Arc System equipped with a 2998 Photodiode Array (PDA) and an ACQUITY QDa Detector. Empower 3 Software was used for data acquisition and processing.

- Sample preparation: 1 gram of the commercially available pesticide formulation was weighed and 9 mL of 50:50 (v/v) acetonitrile/water was added. The resulting mixtures were sonicated for 20 minutes and syringe filtered into an autosampler vial using a 0.2 µm PVDF filter, in preparation for sample analysis.

LC conditions

LC system:	ACQUITY Arc
Separation mode:	Gradient
Column:	CORTECS C_{18}+ 3.0 x 100 mm, 2.7 µm
Solvent A:	Water with 0.1% formic acid
Solvent B:	Acetonitrile
Flow rate:	0.80 mL/min

UV conditions

UV detector:	2998 Photodiode Array (PDA)
PDA detection:	210 to 400 nm
Column temp.:	50 °C
Injection volume:	5 µL
Gradient conditions:	0 min 20% B, 10 min 80% B, 11 min 90% B, 12 min 90, return to initial conditions.

MS conditions

MS system:	ACQUITY QDa
Ionization mode:	ESI+
Capillary voltage:	0.8 kV
Cone voltage:	10 V
Desolvation temp.:	600 °C
Source temp.:	150 °C
MS scan range:	100 to 1000 *m/z*
Sampling rate:	5 Hz

RESULTS AND DISCUSSION

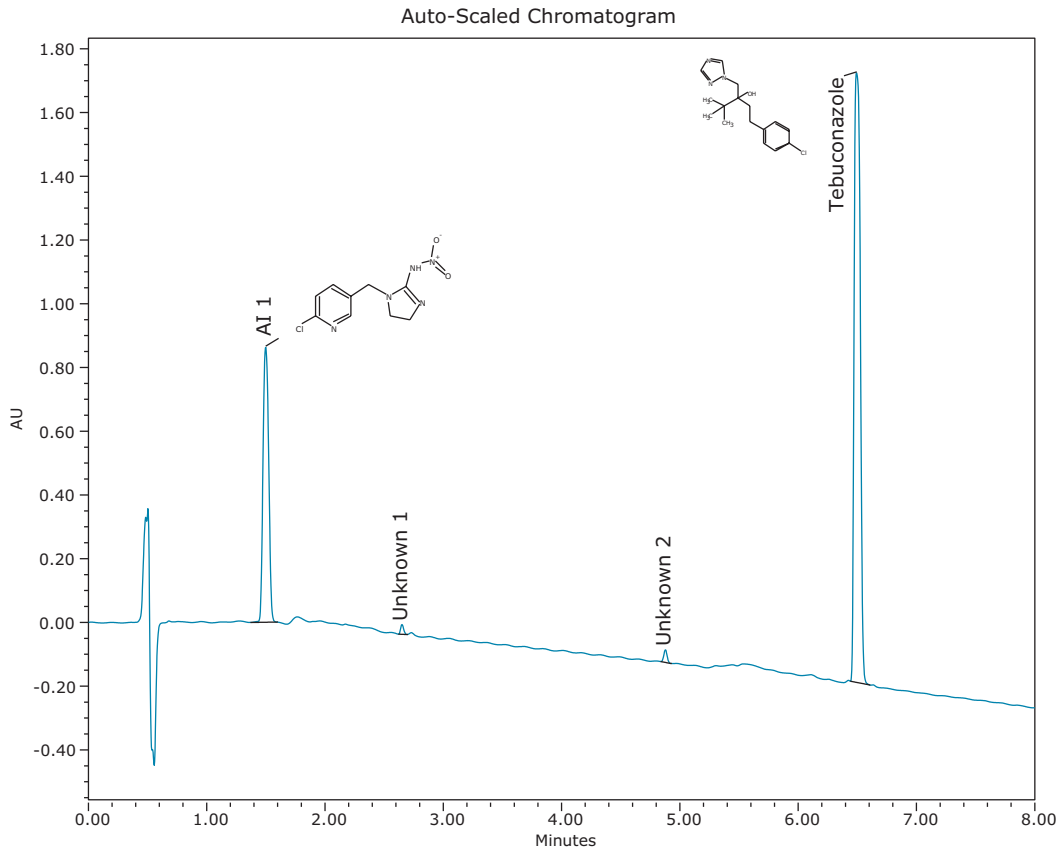
Impurity reporting threshold

The ACQUITY Arc System employs Arc Multi-flow path™ Technology which provides options for selectable dwell volume. This offers increased flexibility for chromatographic separations and maximizes productivity by accommodating 3.0-µm to 5-µm particles for HPLC methods, while also supporting rapid and efficient UHPLC separations using 2.5-µm to 2.7-µm particles.[6] Chromatographic separation of the pesticide formulation sample was performed in UHPLC mode with a CORTECS C_{18}+ Column (3.0 x 100 mm, 2.7-µm solid-core particle technology, part no. 186007402). CORTECS 2.7-µm Columns are compatible on HPLC and UHPLC instrumentation. These columns have high efficiency at HPLC backpressures, resulting in faster analyses with better resolution than current methods using 5 µm, fully porous particles.[7]

Following the analysis of the pesticide formulation and subsequent processing with Empower 3 Software, it was noted that there were two unknown components that exceeded the reporting threshold of 0.1%, which was set in the Empower processing method.[8] The Empower report (Figure 2) shows the ACQUITY Arc UV chromatogram at 220 nm, resulting from the separation of the pesticide formulation sample. The two active ingredients have been identified and the unknown components have been labeled. Beneath the chromatogram, the peak results table displays the component name, area, area%, retention time, impurity response, and the reporting threshold. The UV and MS data suggested that Unknown 1 and Unknown 2 may share common structural features with the tebuconazole AI (Figure 3), therefore an Empower 3 Custom Calculation was employed to calculate the area% of each of the impurities relative to the tebuconazole (Figure 2 table). The tabulated data with the detected impurities highlighted in red makes it easy to interpret which components may require further investigation.

Empower 3 SOFTWARE

Pesticide Formulation Analysis



Peak Results

	Name	RT	Area	%Area	Impurity response	Impurity	Reporting threshold	Impurity response relative to tebuconazole
1	AI 1	1.499	2875440	29.12		No		
2	*Unknown 1*	2.651	54240	0.55	*0.55*	Yes	0.1	*0.79*
3	*Unknown 2*	4.878	76452	0.77	*0.77*	Yes	0.1	*1.11*
4	Tebuconazole	6.494	6868444	69.56		No		
5	*Total impurities*		130692	1.32	*1.32*	Yes		

Figure 2. Empower Software report showing an ACQUITY Arc UV chromatogram at 220 nm resulting from the separation of the pesticide formulation sample. Peak results are shown beneath and impurities exceeding the threshold are highlighted in red. Structures for the known AI's are also displayed in the report.

Combining chromatographic, UV, and mass detector results in a single place in Empower Software can further ease the burden of data interpretation. The Empower Mass Analysis window (Figure 3) provides a single location to associate chromatographic peaks from all of the detectors used in the analysis with their corresponding spectra. The UV chromatogram and spectra are displayed, along with the total ion chromatogram (TIC) and mass spectra with extracted ion chromatograms (XIC) also shown. The spectra from the detected peaks are time aligned and displayed in a window above the chromatograms facilitating rapid data review.

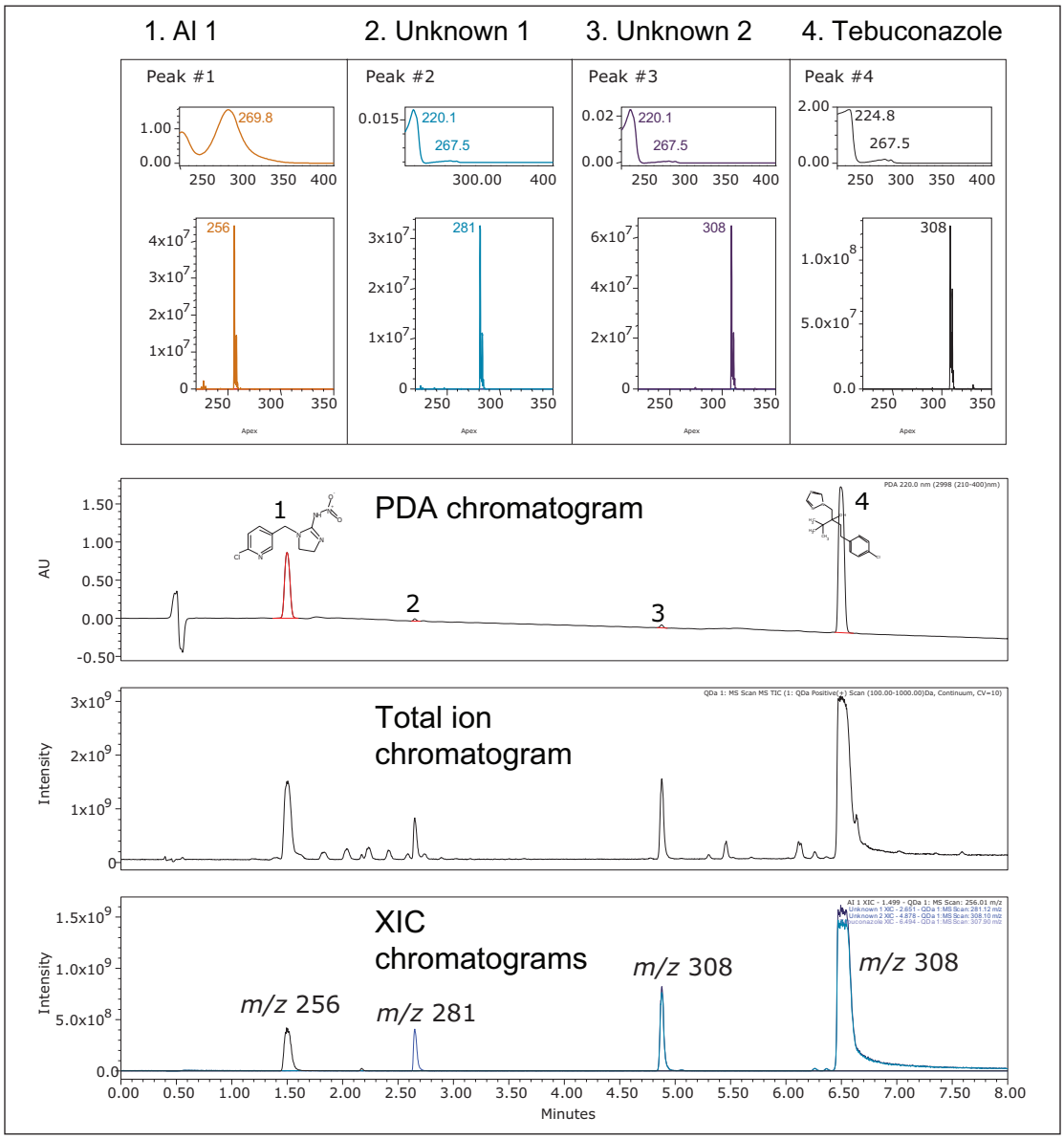


Figure 3. Empower Software Mass Analysis window: UV and MS spectra along with UV and total ion chromatograms (TIC) and extracted ion chromatograms (XIC) can be viewed in a single window.

Interrogation of the data in the Mass Analysis window indicated relationships between Unknown 2 (Peak 3) and the tebuconazole AI (Peak 4). The UV spectra have similar absorption maxima while the mass spectra indicate that tebuconazole, with an $[M+H]^+$ corresponding to *m/z* 308 and Unknown 2 share the same *m/z*. In addition, the isotopic pattern of Unknown 2 is typical of a chlorinated compound (see Figure 4) and is identical to that of the AI. In a single analytical injection, Unknown 2 has been identified as having a similar UV spectrum and the same *m/z* and isotopic pattern as tebuconazole. These results suggest that Unknown 2 is likely an isomer of tebuconazole, and possibly has related structural composition and chemical properties. In addition, the detection sensitivity is greatly improved for the unknown components in the mass chromatogram, especially when the ions corresponding to the peaks of interest have been extracted from the TIC. The increased detection sensitivity provided by the ACQUITY QDa enables improved confidence in the assessment of the data.

The UV maxima of Unknown 1 (Peak 2) showed close similarities when compared with that of Unknown 2 and tebuconazole which may indicate that the impurity is also related to this AI. The *m/z* for Unknown 1 is 281 and the isotopic pattern suggests chlorine in the structure (Figure 4). An Empower Spectrum Index Plot report summarizing the data is shown in Figure 4.

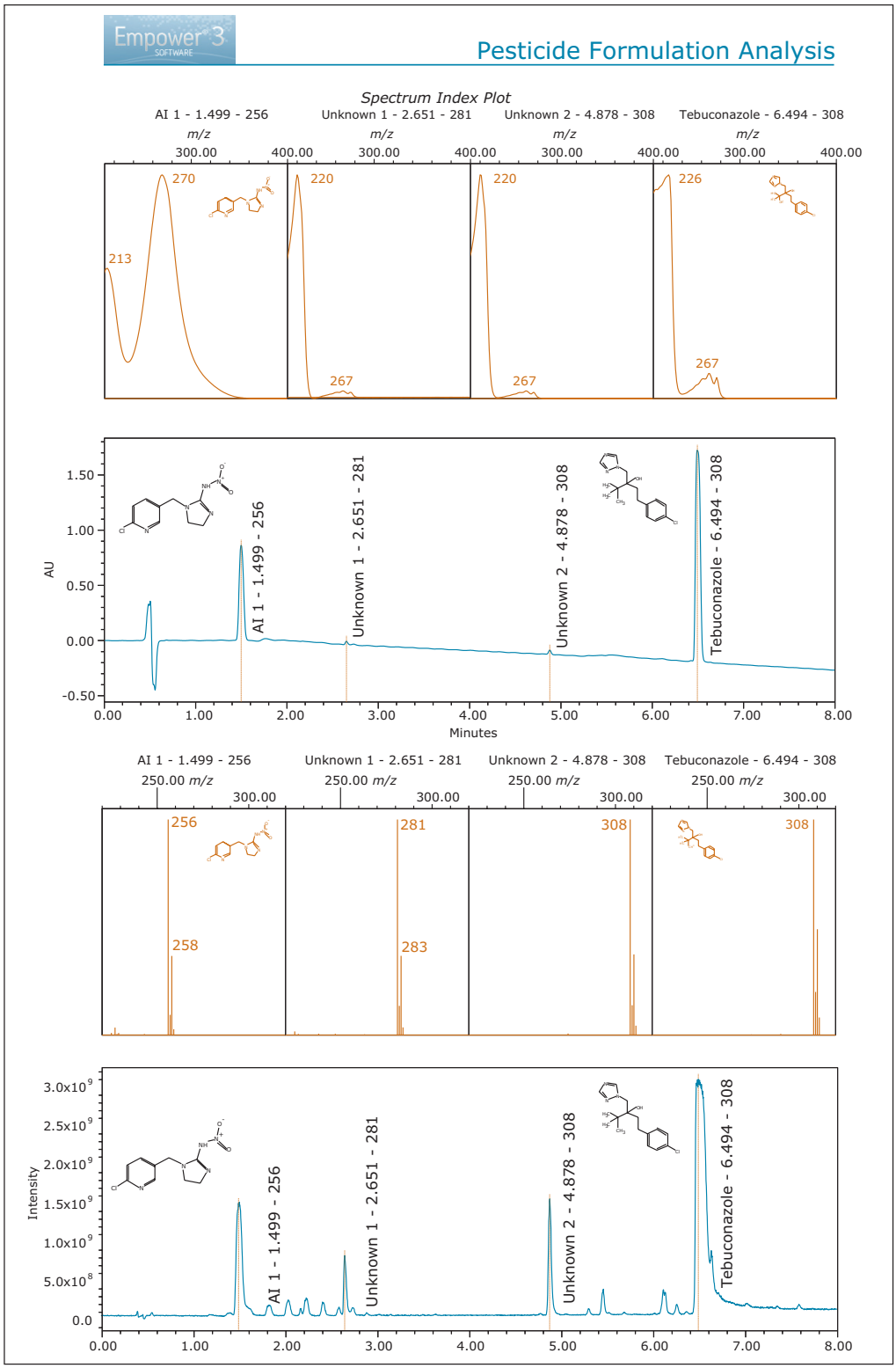


Figure 4. Empower Spectrum Index Plot report showing the ACQUITY Arc UV chromatogram at 220 nm from the analysis of the pesticide formulation, UV spectra of detected peaks are displayed (top), ACQUITY QDa MS scan chromatogram (bottom), and MS spectra for the peaks detected in the UV are also displayed. The structures of the two AI's are shown in the inset.

CONCLUSIONS

- The ACQUITY Arc System provides increased flexibility for chromatographic separations and maximizes productivity by accommodating 3.0-µm to 5.0-µm particles for HPLC methods, while also supporting rapid and efficient UHPLC separations using 2.5-µm to 2.7-µm particles.[6]
- The ACQUITY QDa Detector in combination with the PDA allowed low level components to be detected with increased confidence in the pesticide formulation. The components were identified as having similar optical and structural properties to the triazole fungicide active ingredient present in the formulation sample.
- Empower Software's Mass Analysis window provides a single location to associate the chromatograms and spectra from all of the detectors used in the analysis. The consolidation of this information into one place makes data review and interpretation easy.
- The addition of mass detection as a complementary analytical detection technique enhances confidence in compound detection and identification. With the familiarity of a PDA detector, the ACQUITY QDa Detector provides a cost-effective way to make mass detection routine in laboratories that have previously relied on less selective detectors.
- Empower 3 has numerous features that facilitate the analysis of data. Impurities exceeding a set limit can be automatically flagged. Tailored calculations allow relevant information to be derived quickly which greatly aids in the identification of components that may need further investigation.

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