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Analysis of Disperse Dyes Using the ACQUITY Arc System with PDA and Mass Detection, and Empower Software

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APPLICATION BENEFITS

- Enhanced confidence in the profiling of impurities using PDA and mass detection.
- Ease of use with single point control via Empower® 3 Software.
- Dual-flow paths to emulate HPLC and UHPLC separations.

WATERS SOLUTIONS

ACQUITY® Arc™ System

2998 Photodiode Array (PDA) Detector

ACQUITY QDa® Detector

XBridge® C_{18} Column

Empower 3 CDS Software

KEY WORDS

Disperse dyes, consumer products, textile, impurity identification, mass detection

INTRODUCTION

Disperse dyes are low molecular weight synthetic dyes. The structure of the dyes can often contain azo or anthraquinone functional groups.[1] The primary application of disperse dyes is in consumer products such as textiles, paper, toys etc. Several of the dyes have been found to induce an allergic response as a result of prolonged exposure to the skin.[2] The presence of azo groups in the structure of some dyes provides the possibility for them to be converted to potential or known carcinogenic aromatic amines.[2]

The existence of these dyes in consumer products has led to increased awareness of the potential harmful effects to consumer health. Legislation controlling the use of several of these dyes was introduced in Germany in 1996. This led to the development of the DIN 54231 standard procedure which describes a method for the analysis of disperse dyes that employs high performance liquid chromatography (HPLC) or thin layer chromatography (TLC) with either ultraviolet (UV), mass spectrometry (MS), or densitometry detection for the analysis of the dyes.[3-5]

In this application note, we present the analysis of nine disperse dyes (Figure 1) using the standard DIN 54231 procedure with a combination of UV and mass detection, and a dual-flow path liquid chromatography system capable of emulating HPLC or UHPLC separations.[6] The inclusion of the mass detector allowed increased information to be derived from the analysis including confirmation of impurity peaks in specific dye samples. The detection limit when measured using the disperse blue 1 dye standard is specified as 0.7 mg/L in the DIN 54231 method. Using Waters® ACQUITY Arc System and the ACQUITY QDa Detector, the detection limit achieved significantly surpassed the specified detection limit for all compounds evaluated.

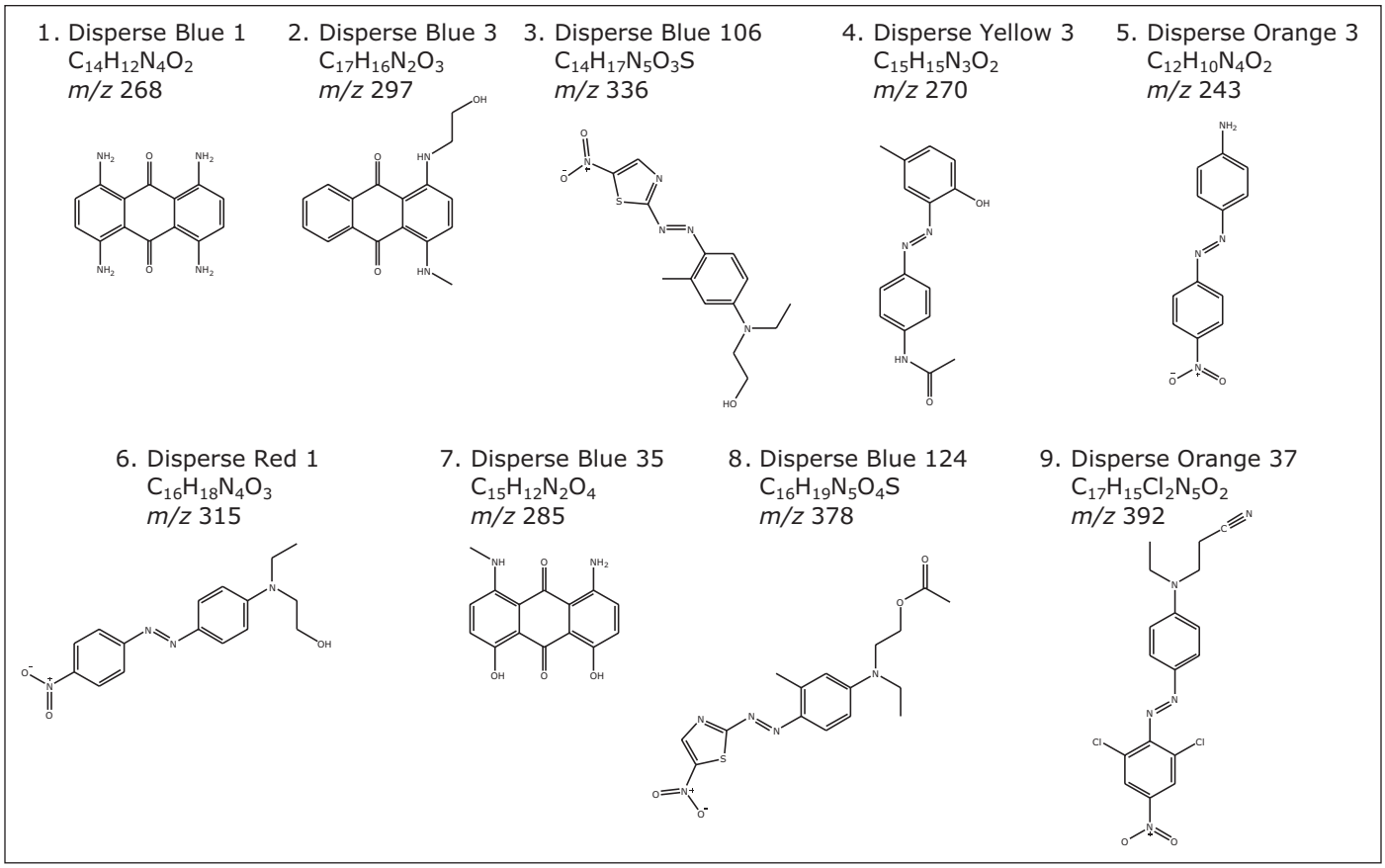

Figure 1. Empirical formulas, structures, and m/z for the disperse dyes used in this study.

EXPERIMENTAL

Instrumentation and software

All separations were performed on the ACQUITY Arc System equipped with a 2998 Photodiode Array (PDA) Detector and positive ion electrospray mass spectrometry (MS) using the ACQUITY QDa Detector. Empower 3 Software was used for data acquisition and processing.

Sample preparation

The dye standards were dissolved in methanol and sequentially diluted in preparation for sample analysis.

LC conditions

HPLC method (DIN 54231)

LC system:	ACQUITY Arc
Separation mode:	Gradient
Column:	XBridge C_{18}, 2.1 x 150 mm, 5 µm
Solvent A:	Ammonium acetate 10 mmol pH 3.6
Solvent B:	Acetonitrile
Flow rate:	0.30 mL/min
PDA detection:	210 to 800 nm
Column temp.:	30 °C
Injection volume:	5 µL
Analysis time:	30 min
Gradient conditions:	0 min 40% B, 7 min 60% B, 17 min 98% B, 24 min 98% B, return to initial conditions.

MS conditions

MS system	ACQUITY QDa
Ionization mode:	ESI +
Capillary voltage:	1.2 kV
Cone voltage:	10 V
Desolvation temp.:	600 °C
Source temp.:	120 °C
MS scan range:	100 to 600 *m/z* and Selected Ion Recording (SIR)
Sampling rate:	5 Hz

Figure 2 shows a PDA chromatogram at 240 nm resulting from the separation of a mixture of nine disperse dye standards (lower trace), and the superimposed SIR channels (top trace) obtained using a 2.1 x 150 mm, 5-µm XBridge C_{18} Column, (Part no. 186003110).

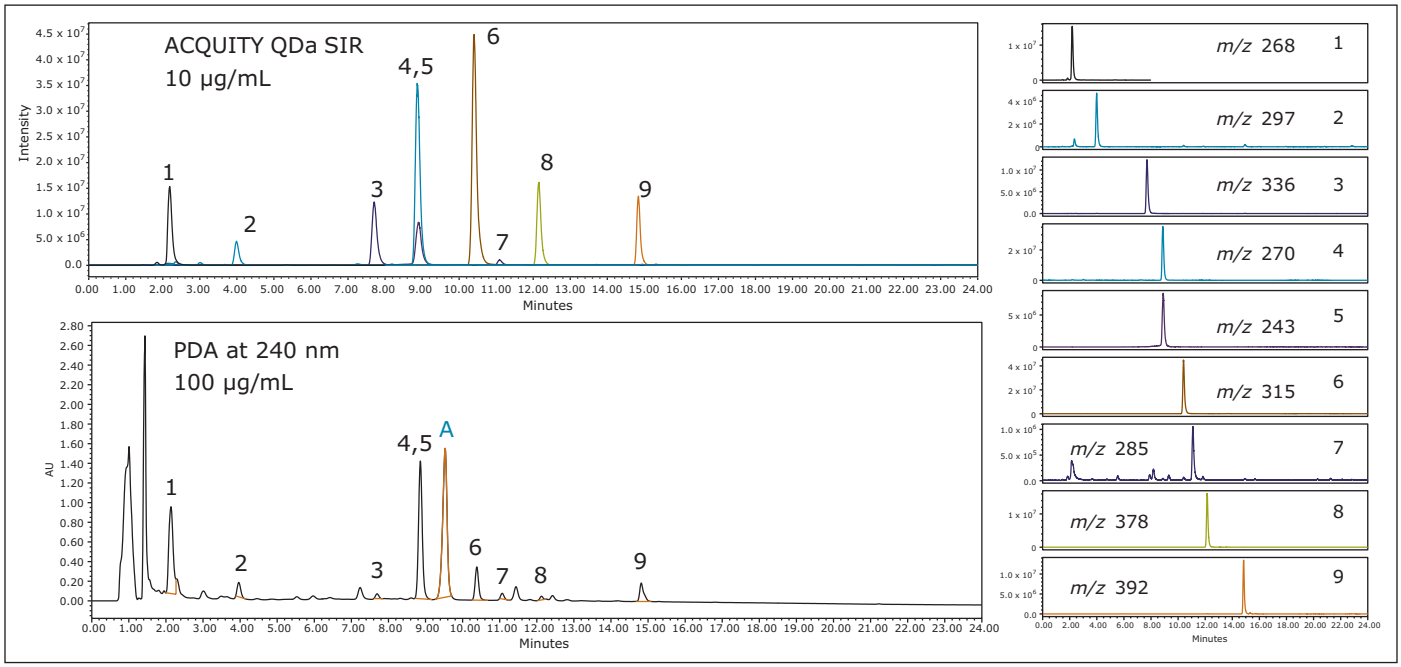

Figure 2. ACQUITY Arc chromatogram from the separation of nine disperse dye standards (100 µg/mL, 5 µL injection) at 240 nm using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column (lower). The superimposed (top) and the individual stacked (right) SIR channel chromatograms (10 µg/mL, 5 µL injection) are also shown.

Note that there is a coelution of the chromatographic peaks resulting from disperse yellow 3 (peak 4), and disperse orange 3 (peak 5) which makes accurate detection by UV alone challenging. Chromatographic separation of the components would be required for accurate detection if UV was to be used which would extend the method development time. The components have different *m/z* ratios, which enabled independent detection using the ACQUITY QDa despite the coelution, as can be seen from the stacked individual SIR chromatograms shown in Figure 2. Detection sensitivity was significantly improved using the mass detector.

Impurity analysis

A prominent peak (peak A) was detected in the PDA data at a retention time (t_R) of 9.5 minutes. This signal was absent from the SIR channels as the specific *m/z* for this unknown component was not monitored in the experimental method. An MS full scan experiment was performed simultaneously with the PDA detector making it possible to determine the mass spectra as well as the UV spectra for all components in the mixture (Figure 3).

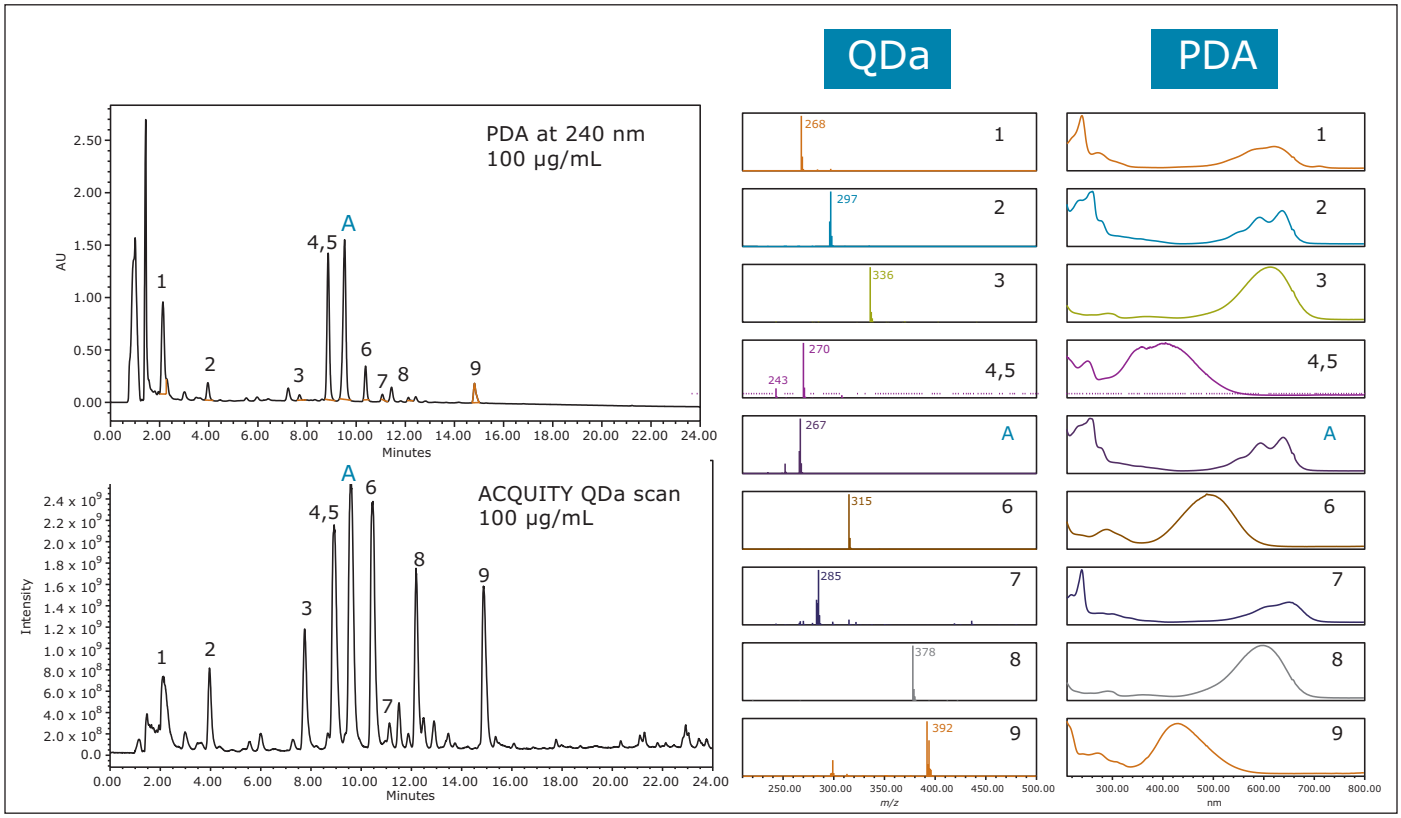

Figure 3. ACQUITY Arc chromatograms from the separation of nine disperse dye standards at 240 nm (top) (100 µg/mL, 5 µL injection) and QDa MS scan (100–600 m/z) (bottom) using the DIN 54231 standard method and an XBridge C_{18}, 2.1 x 150 mm, 5.0-µm Column. The MS and UV spectra are also shown.

The MS spectra for the unknown component A showed a large spectral peak with *m/z* 267. In addition the UV spectra of peak 2 which corresponds to disperse blue 3 and that of unknown peak A had similar features indicating that they may share common structural characteristics. A standard solution containing only disperse blue 3 which had a dye content of 20% was analyzed (Figure 4). The Mass Analysis window from Empower Software allowed rapid confirmation of the identity of disperse blue 3 (*m/z* 297) by displaying both the UV and mass spectra simultaneously. The mass spectrum for unknown peak A indicates that the base peak for this component is *m/z* 267 which matched the previous analysis of the mixture. In addition the t_R and the UV spectra were the same in both analyses. A second unknown component with a t_R of 11.4 minutes, labeled B, with an *m/z* 254 was also detected in the analysis of the disperse blue 3 dye standard. The ACQUITY QDa and PDA data provided complementary information which allowed us to conclude that the impurity A previously detected in the mixture of dyes originated from the disperse blue 3 standard.

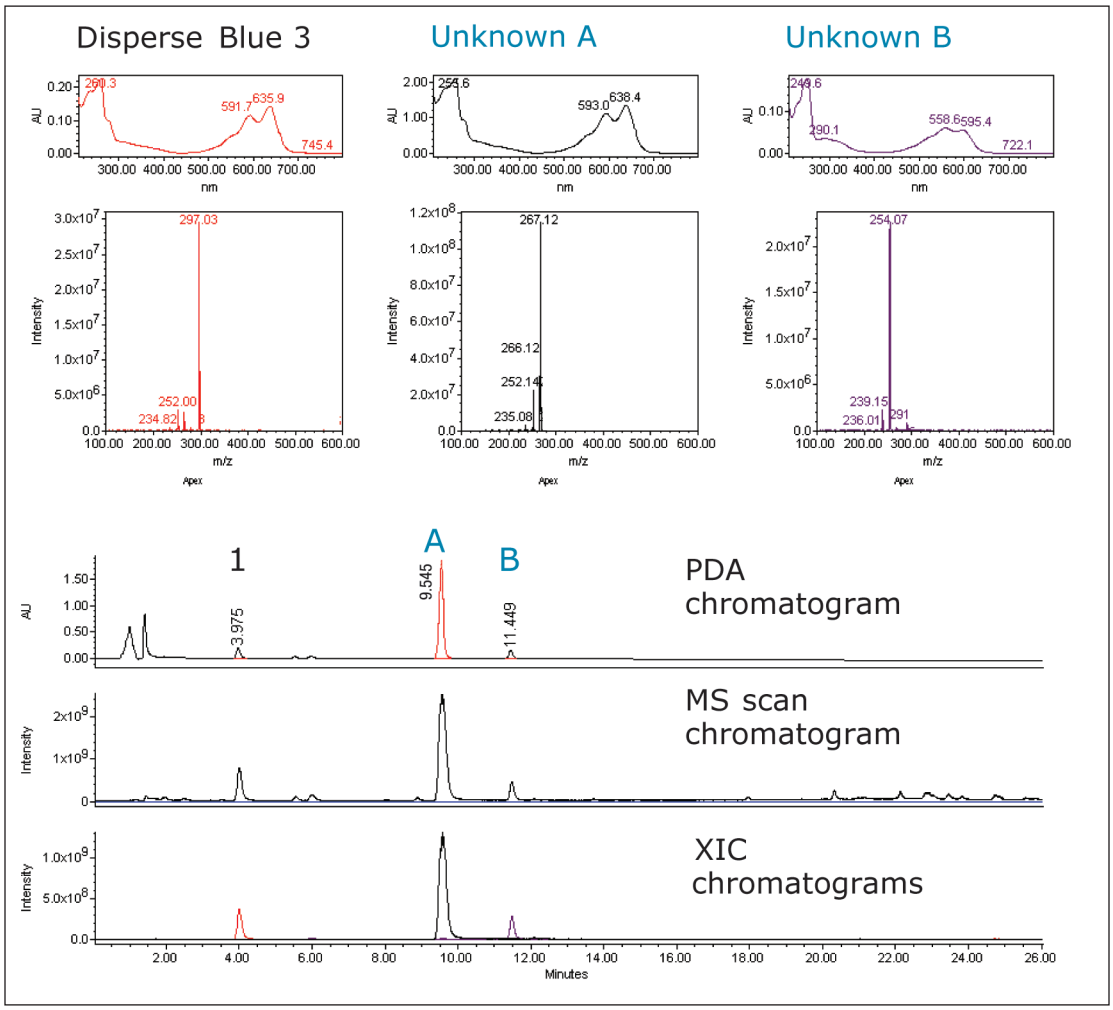

Figure 4. Empower Software's Mass Analysis window showing UV and MS spectra (top). ACQUITY Arc -, PDA, MS scan (100–600 m/z) and superimposed XIC chromatograms of a single standard of disperse blue 3 using the DIN 54231 standard method.

CONCLUSIONS

The addition of mass detection as a complementary analytical detection technique enhances confidence in compound detection and identification. Co-eluting components with different *m/z* ratios can be reliably analyzed using mass detection. The detection limits required for the DIN method can be surpassed for all compounds using the described analytical methodology. The presence of both PDA and mass detection helped confirm that an impurity detected during method development originated in the disperse blue 3 standard. Thus the addition of mass detection acts as a complementary technique for impurity analysis.

The ACQUITY Arc System provides 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 to -2.7 µm particles.[6]

References

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