ANALYSIS OF DISPERSE DYES USING HPLC WITH PDA AND MASS DETECTION

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INTRODUCTION

Disperse dyes are low molecular weight synthetic dyes. The primary application of disperse dyes is in consumer products such as textiles, paper, toys, industrial adhesive glue and cleaning products, agricultural seed colorants, cosmetic and personal care products.[1] Several of the dyes have been found to induce an allergic response due to prolonged exposure to the skin.[1]

The existence of the dyes in consumer products has led to increased awareness of the potential harmful effects to consumer health. Exposure to some of the dyes has been restricted. Legislation controlling the use of several dyes was introduced in Germany in 1996. This led to the development of the DIN 54231 standard procedure which describes analytical methodology for the analysis of disperse dyes.[2]

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.[3]

Co-eluting components with different *m/z* ratios that cannot be analysed by UV detection alone 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 from the disperse blue 3 standard.

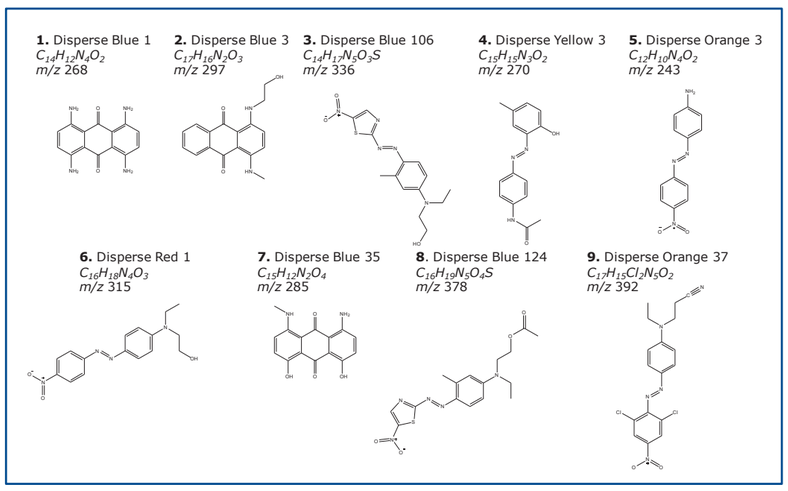


Figure 1. Empirical formulas, structures, and m/z for the disperse dyes used in this study.

METHODS

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
Separation mode: Gradient
Column: XBridge C18, 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
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 +
MS scan range: 100 to 600 *m/z* and Selected Ion Recording (SIR)
Sampling rate: 5 Hz

RESULTS AND DISCUSSION

- 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 C18 Column.
- 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.
- 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.

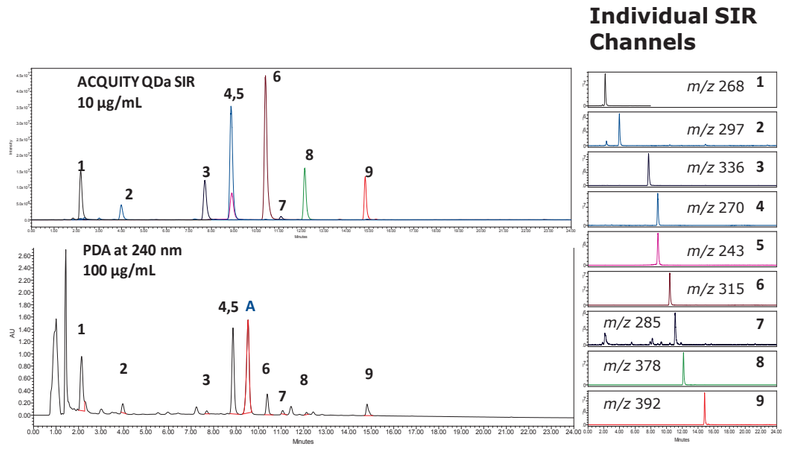


Figure 2. ACQUITY Arc chromatogram from the separation of nine disperse dye standards (100 µg/mL, 5 µL injection) at 240 nm using the DIN 54231 standard method and an XBridge C18 , 2.1 x 150 mm, 5.0-µm Column (lower). The superimposed (top) and the individual stacked (right) SIR channel chromatograms (10 µg/mL, 5 µL injection) are also shown.

Impurity analysis

- A prominent unknown component (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 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).

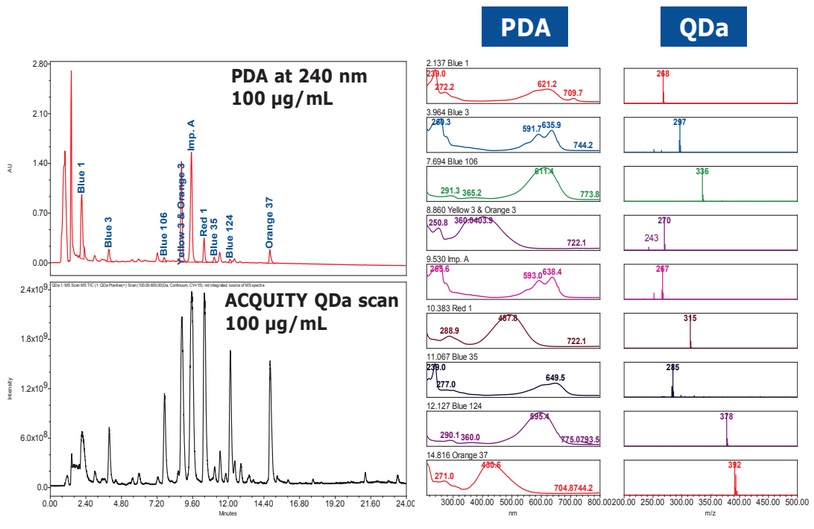
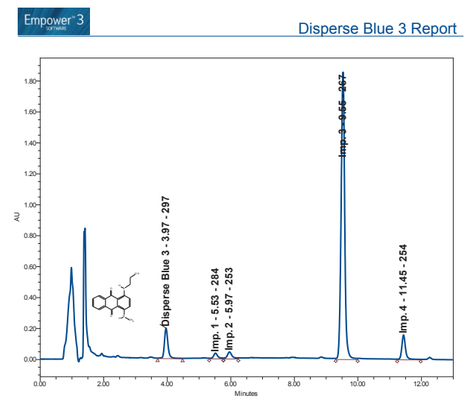


Figure 3. ACQUITY Arc chromatograms from the separation of nine disperse dye standards at 240 nm (top) (100 µg/mL, 5 µL injection) and QDa MS scan (100–600 m/z) (beneath) using the DIN 54231 standard method and an XBridge C18 , 2.1 x 150 mm, 5.0-µm column. The MS and UV spectra are also shown.

- The MS spectra for the unknown component A showed a large spectral peak with *m/z* 267. The UV spectra of disperse blue 3 (Figure 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). Several impurities were detected in this analysis and were labeled with respect to elution order (Imp. 1-4). Component A from the previous analysis corresponds to Imp 3. The mass spectrum for Imp 3 indicated that the base peak for this component was also *m/z* 267 which matched the previous analysis of the mixture.
- Empower software was used to flag impurities peaks that exceeded the 0.1 Area% level. The impurity response of the component with *m/z* 267 (Imp 3, Figure 4) relative to the disperse blue 3 standard was calculated using a custom calculation (Figure 4 table).
- The ACQUITY QDa and PDA data provided complementary information which allowed us to conclude that the compound A/Imp.3 previously detected in the mixture of dyes originated from the disperse blue 3 standard.



	Peak Name	RT	Area	% Area	Impurity	Impurity Response	Reporting Threshold	Impurity Response to Disperse Blue 3
1	Disperse Blue 3	3.97	1443465	8.03	No			
2	*Imp. 1*	5.53	311993	1.74	Yes	*1.74*	0.1	
3	*Imp. 2*	5.97	436838	2.43	Yes	*2.43*	0.1	
4	*Imp. 3*	9.55	14529767	80.83	Yes	*80.83*	0.1	*1006.59*
5	*Imp. 4*	11.45	1254713	6.98	Yes	*6.98*	0.1	
6	*Total Impurities*		16533312	91.97	Yes	*91.97*		

Figure 4. Empower Software report showing an ACQUITY Arc UV chromatogram at 240 nm resulting from the separation of the disperse blue 3 standard. Peak results are shown beneath, and impurities exceeding the threshold are highlighted in red. Structure for disperse blue 3 is also displayed in the report.

CONCLUSION

- 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.[3]

References

1. J Garcia-Lavandeira, E Blanco, C Salgado, R Cela. Fast throughput, highly sensitive determination of allergenic disperse dyes in textile products by use of sample composition. (2010) *Talanta*, 82: 261–269.
2. German Institute for Standardisation (DIN). Textiles-Detection-Detection of Dispersed Dyestuffs. DIN 54321:2005.
3. Waters ACQUITY Arc System Brochure, no. 720005393en, June, 2015.

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