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## INTRODUCTION

Ink-jet printing applies not only to office documents but also to such diverse applications as kayaks, beverage cans, cardboard cartons and textiles. Ink-jet inks are complex mixtures and those that use organic dyes can also contain water, organic solvents, bactericides/fungicides, binders and other additives depending on the end use. It is essential that ink-jet inks are formulated for the system or process and surface to be printed. High contrast colors, lightfastness, and good adhesion properties are typical requirements for ink.

This application note describes the comparison of ink-jet formulas from generic and brand printer cartridges utilizing the Waters® ACQUITY UPLC™/UV system coupled with the Waters ZQ™ mass detector. The ink dyes and the polymeric surfactant ingredients were separated and detected in two minutes using the ACQUITY UPLC system. This is 10 times faster than traditional RP-HPLC (reversed phase), which resolves dyes<sup>1-4</sup> or polymers<sup>5</sup> of similar chemical structures in approximately 20 minutes.

The resolution, sensitivity and speed enhancements from UPLC combined with UV and MS detection represents a powerful method for analyzing ink dyes and evaluating the photo-stability of dye components.<sup>6-9</sup> The focus of the previous application note in this series<sup>9</sup> was on the ACQUITY UPLC system with UV detection only. This note describes a 2-minute method to separate ink-jet dyes and polymer ingredients using the UPLC™/UV/ ZQ single quadrupole MS system.

The ability to quickly and unambiguously characterize ink formulas can facilitate workflow in production such as certifying lot-to-lot variation and product troubleshooting. Central analytical and research labs will find this approach useful for evaluating competitive products and in patent infringement defense scenarios.

## EXPERIMENTAL

### Sample Preparation

The ink-jet solutions (0.5-1 µL) were extracted from ink-jet printer cartridges by pipette and diluted with 1-2 mL of D.I. water. The sample solutions were filtered with 25 mm GHP Acrodiscs filters (WAT 200514) and placed in Max Recovery vials (186000327c) for UPLC analysis.

### UPLC System Operation Conditions

System:	ACQUITY UPLC with ACQUITY UV detector and Waters ZQ 2000		
Software:	Waters Empower™ Software		
Column:	ACQUITY UPLC BEH C <sub>18</sub> 2.1x 50 mm		
Column Temp:	50 °C		
Weak Wash :	95:5	Water: CH <sub>3</sub> CN	(500 µL)
Strong Wash:	50:50	Water: CH <sub>3</sub> CN	(300 µL)
Seal Wash:	90:10	Water: CH <sub>3</sub> CN	(5 min)
Mobile Phase A:	10 mM NH <sub>4</sub> HCO <sub>3</sub> , pH 9.8		
Mobile Phase B:	CH <sub>3</sub> CN		
Linear Gradient:	10% B to 65% B in two minutes		
Flow Rate:	1 mL/min		
Injection:	5 µL		
Detection:	UV absorbance (at 400 nm)		
Sampling rate:	20 pts/s		
Filter Response:	0.1 s		

### ZQ Parameters

Probe:	ES+	ES capillary (kV):	3.2
Cone (V):	40	Extractor (V):	2
RF Lens (V):	0.5	Multiplier for ES+:	500
Probe:	ES-	ES capillary (kV):	-3.0
Cone (V):	-30	Extractor (V):	-2
RF Lens (V):	-0.5	Source Temp (°C):	140
Desolvation Temp (°C):	450	Cone Gas flow (L/Hr):	50
Desolvation Gas (L/Hr):	800	LM Resolution:	15
HM resolution:	15	Ion Energy:	0.3
Multiplier for ES-:	650	Scan Range:	150 to 1000 Da
Scan time:	0.22s	Inter-scan delay:	0.05s

*Note: Retention time of UV was offset 0.011 minutes to match TIC chromatograms. ES- and ES+ modes were run separately. UV chromatograms of both runs were used to support the experiments.*

## RESULTS AND DISCUSSIONS

Yellow ink-jet inks from three different vendors (brand and generic) were separated using the ACQUITY UPLC system with a two-minute linear gradient method and ammonium bicarbonate pH 9.8 and acetonitrile as the mobile phases. The ZQ single quadrupole mass detector combined with UV detection were used for peak tracking and identification of dye components. Figures 1, 2, and 3 show the UV absorbance, negative and positive ES TIC (electrospray total ion current) chromatograms of the ink-jet inks.

The UV chromatograms show two major dye components in the Vendor 1 ink at 0.40 (**d1**) and 0.88 (**d2**) minutes; one major dye component in the Vendor 2 ink at 0.88 (**d2**) minutes; one major dye component in the Vendor 3 ink at 0.43 (**d3**) minutes. The ES<sup>-</sup> TIC chromatograms matched the peaks observed with the UV detector. The ES<sup>+</sup> TIC chromatograms showed peaks not observed in either the UV or the ES<sup>-</sup> TIC chromatograms illustrating the value of combining UV detection with positive/negative ion MS.

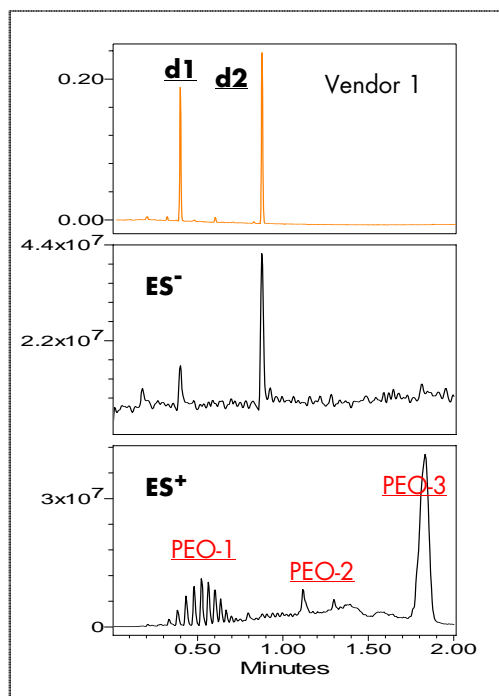


Figure 1. UV (400 nm), negative and positive ES TIC chromatograms of yellow ink-jet ink from Vendor 1.

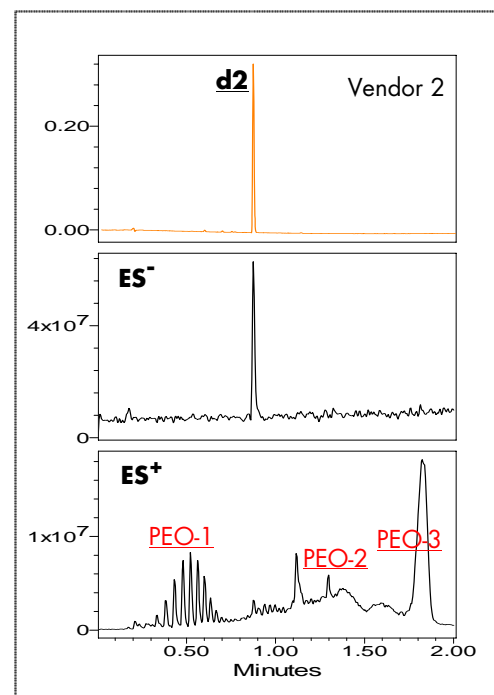


Figure 2. UV (400 nm), negative and positive ES TIC chromatograms of yellow ink-jet ink from Vendor 2.

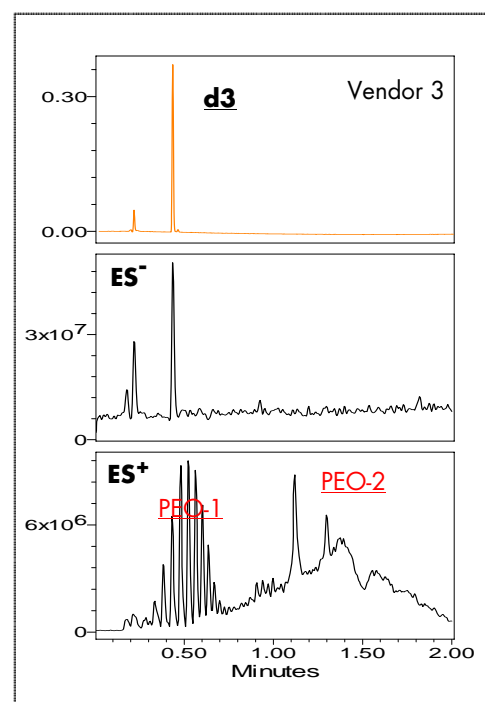


Figure 3. UV (400 nm), negative and positive ES TIC chromatograms of yellow ink-jet ink from Vendor 3.

Figures 4 and 5 show negative and positive ion mass spectra of dye components **d1**, **d2** and **d3**. The mass spectrometry data enabled the unambiguous differentiation among Vendors 1, 2 and 3.

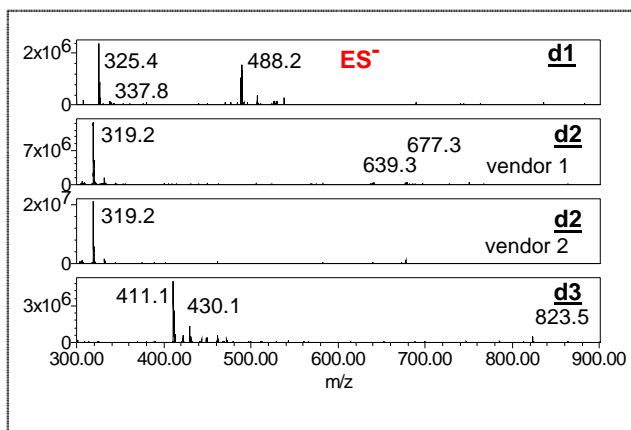


Figure 4. Negative ion mass spectra at retention time (min): 0.39-0.41 (**d1**), 0.87-0.90 (**d2**), 0.42-0.45 (**d3**).

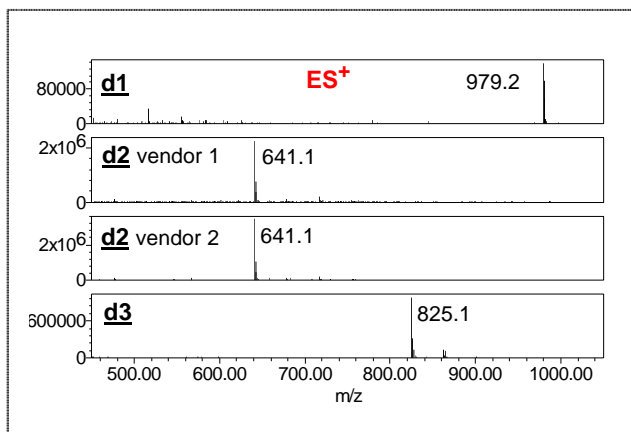


Figure 5. Positive-ion mass spectra at retention time (min): 0.39-0.41 (**d1**), 0.87-0.90 (**d2**), 0.42-0.45 (**d3**).

The interpretation of negative-ion mass spectra of acidic ink-jet dyes is straightforward.<sup>4,10</sup> The molecular mass of the acidic dye, M, can be determined from the series of  $[M-nH]^-$  ions, where n is the number of charges carried by the ion and H is hydrogen. For salt adducts  $[M-(n+x)H+xS]^-$ , where the maximum value of n or (n+x) is equal to the total number of acidic groups, S is the atomic weight of adduct species (23 for Na, 39 for K, etc.).

Although the positive-ion peak intensity of ink dyes is weaker than the negative-ion intensity, mass spectra can be extracted from the ES+ TIC chromatograms. The m/z ratios of positive ions are useful for further confirming the molecular mass of the dye calculated using the m/z ratios of a series of negative ions.

The molecular mass of **d1**, 978 Da, is calculated from the m/z of negative ions 325 ( $[M-3H]^{3-}$ ), and 488 ( $[M-2H]^{2-}$ ); 10 and confirmed by the m/z of potassium adduct 337.8 ( $[M-4H+K]^{3-}$ ), and the m/z of M+H ion at 979 ( $[M+H]^+$ ). The molecular mass of **d2**, with 640 Da is obtained from m/z of 319 ( $[M-2H]^{2-}$ ), and m/z 639 ( $[M-H]^{1-}$ ); 10 and verified by the m/z of potassium adduct 677 ( $[M-2H+K]^{1-}$ ), and the m/z of M+H ion at 641 ( $[M+H]^+$ ). The molecular mass of **d3** with 824 Da, is determined from the m/z of 411 ( $[M-2H]^{2-}$ ) and, 823 ( $[M-H]^{1-}$ ); 10 and validated by the m/z of potassium adduct 430 ( $[M-3H+K]^{2-}$ ), and the m/z of M+H ion at 825 ( $[M+H]^+$ ).

Although **d1** and **d3** have similar retention times, 0.40 and 0.43 minutes, respectively, the MS data clearly indicate different species. The mass spectra of ES negative and positive ions unambiguously confirm the presence of **d2** in the formulas of both Vendors 1 and 2.

The inks of Vendors 1 and 3 have no common dye components. These results indicate the utility of applying the UPLC/UV/ZQ system in dye related industries for fast screening of imitation products, competitive analysis and patent infringement.

The ES positive-ion mass spectra of **PEO-1**, **PEO-2**, and **PEO-3** consist of several adjacent ion envelopes each based on 44 Da spacing. This indicates poly(ethylene oxide) based polymers with different molecular weight distributions, end groups, and branches.

The positive-ion TIC chromatograms of Vendors 1 and 2 have nearly identical peaks (Figures 1 and 2). A notable difference is that the relative peak intensities in **PEO-3** in the Vendor 1 ink are larger than those in the Vendor 2 ink. Vendor 3 contains all the peak components of Vendors 1 and 2 except **PEO-3** (Figure 3).

Figure 6 shows the extracted mass spectra of eight **PEO-1** peaks with retention times from 0.38 to 0.67 minutes. The mass spectra of each peak contained two or three major ions that are recognized as  $[H(OCH_2CH_2)_N OH + H]^+$  ions accompanied by salt adducts,  $[H(OCH_2CH_2)_N OH + NH_4]^+$ , or  $[H(OCH_2CH_2)_N OH + K]^+$ , where N is 7 to 14. For example, the mass spectrum of the peak at 0.67 minutes has ions at  $m/z$  635.3, 652.3, and 673.3 (Da), which are consistent with  $[H(OCH_2CH_2)_{14} OH + H]^+$ ,  $[H(OCH_2CH_2)_{14} OH + NH_4]^+$ , and  $[H(OCH_2CH_2)_{14} OH + K]^+$ .

These results for the separation of polymers with the UPLC /UV/ZQ system are 10 times faster than similar results reported with conventional RP-HPLC and provide molecular weight and polymer composition information.<sup>5</sup>

## CONCLUSION

The Waters ACQUITY UPLC combined with UV and ZQ MS detectors provides an information-rich, time-efficient tool for identifying and differentiating the dyes and polymeric components in ink-jet ink cartridges.

There are multiple benefits in combining MS and UV detection after UPLC separation for ink-jet ink analyses. The retention time of UV (400nm) chromatogram peaks identifies the dye components; whereas, the extracted mass spectra of negative-ion TIC chromatograms reveal the molecular weight of dyes and the extracted mass spectra of positive-ion chromatograms reveal polymer structures that were undetected by UV.

Potential applications are competitive deformation, imitation product verification and patent infringement litigation, batch-to-batch product quality control and troubleshooting. The resolution power and separation speed for polymers and dyes will prove useful for ink-jet ink developers and producers.

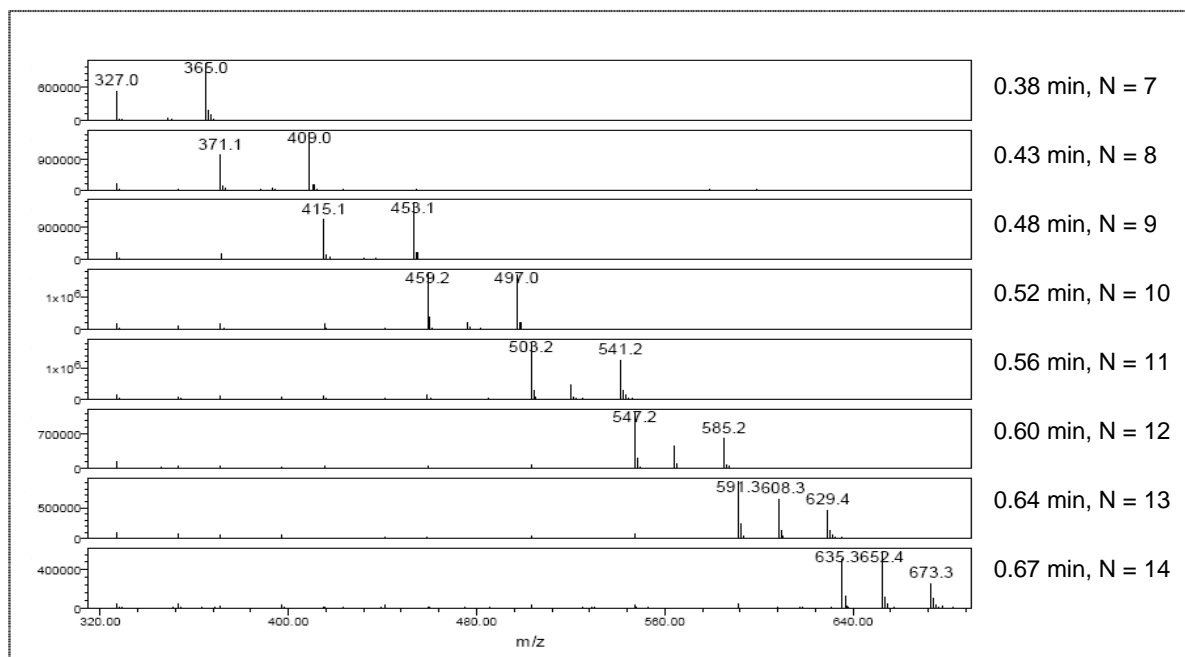


Figure 6. Positive-ion mass spectra of **PEO-1** peaks at retention time 0.38, 0.43, 0.48, 0.52, 0.56, 0.60, 0.64, 0.67 (min).

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10.  $M$  (molecular mass of analyte) =  $m_1n_1 + n_1$  (eq. 1),  
 $n_1 = (m_2 + 1)/(m_1 - m_2)$  (eq. 2), in which the negative  $m_2$  ion is at a lower  $m/z$  value than  $m_1$ , and  $n_1$  is the number of charges carried by the negative  $m_1$  ion.

To calculate molecular mass of **d1** by using equation 2. we may define the following:  
 $m_1 = 488$ ;  $m_2 = 325$ , and  
 $n_1$  is given by  $(325 + 1)/(488 - 325) = 2$   
 By using equation 1,  $M$  of **d1** is given by  
 $488 \times 2 + 2 = 978$ .

To calculate molecular mass of **d2** by using equation 2. we may define the following:  
 $m_1 = 639$ ;  $m_2 = 319$ , and  
 $n_1$  is given by  $(319 + 1)/(639 - 319) = 1$   
 By using equation 1,  $M$  of **d2** is given by  
 $639 \times 1 + 1 = 640$ .

To calculate molecular mass of **d3** by using equation 2. we may define the following:  
 $m_1 = 823$ ;  $m_2 = 411$ , and  
 $n_1$  is given by  $(411 + 1)/(823 - 411) = 1$   
 By using equation 1,  $M$  of **d3** is given by  
 $823 \times 1 + 1 = 824$ .

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