Determination of EU-Banned Disperse Dyes by LC/MSD TOF

Application

Environmental

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Abstract

Accurate mass measurements are used to both confirm the presence of a compound and help identify unknowns. Furthermore, high-mass accuracy enables the user to generate very narrow extracted ion chromatograms, enabling excellent quantitation. This application note describes the use of the Agilent liquid chromatograph/mass selective detector time-of-flight (LC/MSD TOF) instrument to identify and quantitate the disperse dyes used in textiles. This is regulated by the European Commission (EC), which is the trade regulatory arm of the European Union (EU).

Introduction

Disperse dyes were originally developed for the dyeing of cellulose acetate. They are substantially water insoluble. The dyes are finely ground in the presence of a dispersing agent, then sold as a paste or spray, dried and sold as a powder. They can also be used to dye nylon, triacetate, polyester, and acrylic fibers.

The general structure of disperse dyes is small, planar, and non-ionic, with attached polar functional groups like -NO_2 and -CN. The shape makes it easier for the dye to slide between the tightly-packed polymer chains, and the polar groups improve both the water solubility and the dipolar bonding between dye and polymer, affecting the color of the dye. However, their small size means that disperse dyes are quite volatile, and tend to sublime out of the polymer at sufficiently high temperatures.

Increasingly, liquid chromatography/mass spectrometry (LC/MS) is being used for the analysis of many polar dyes of the EU Directives, as passed down by the European Commission (EC), and is rapidly becoming an accepted technique in dye residue analysis for regulated monitoring. In particular, LC/MS works well on the new families of disperse dyes that are polar and thermally labile. Thus, LC/MS methods are often preferred over the older, established GC/MS techniques, which have often required derivatization for analysis or the liquid chromatography/ultraviolet (LC/UV) methods, which are less sensitive and less selective.

As a result, LC/MS is an emerging technology for the analysis of dyes, and here we present the results of using the Agilent time-of flight instrument, or LC/MSD TOF, for the accurate mass and high resolution analysis of dyes.

Experimental

The work undertaken in this study was performed on an Agilent 1100 LC system consisting of a binary pump, autosampler, thermostatted column compartment, and the LC/MSD TOF. The compounds analyzed are shown in Table 1.

Table 1. The 20 Disperse Dyes Studied, Including Chemical Abstract Services Number (in brackets), Chemical Structure, and Theoretically Calculated Exact Mass

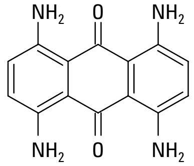
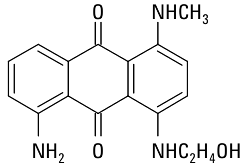
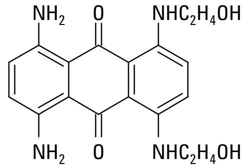
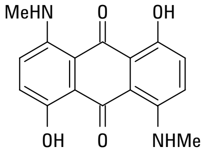
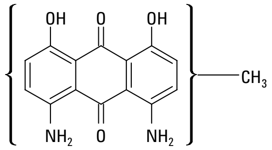
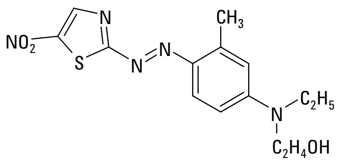
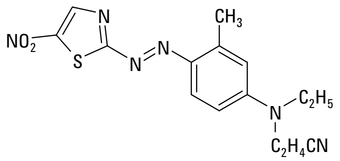
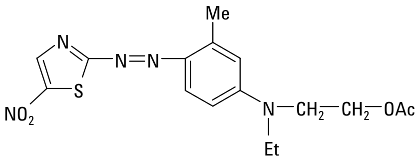
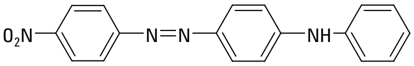
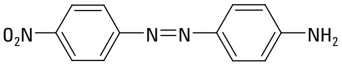
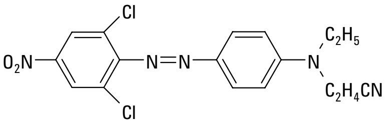
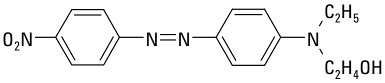
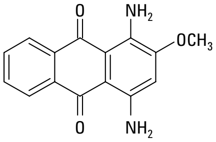
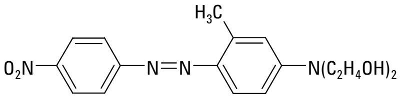
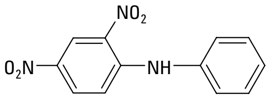
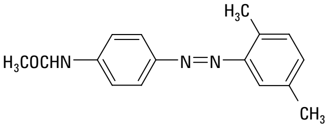
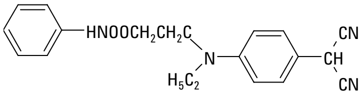
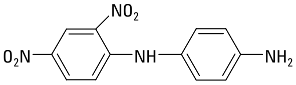
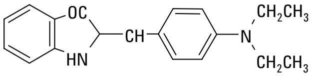
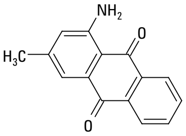
	Disperse dye	Molecular formula	Structure	Mono-isotopic mass
1	Disperse Blue 1 [2475-45-B]	$C_{14}H_{12}N_4O_2$		2.68.2744
2	Disperse Blue 3 [2475-46-9]	$C_{17}H_{16}N_2O_3$		296.325
3	Disperse Blue 7 [3179-90-6]	$C_{18}H_{18}N_2O_6$		358.35
4	Disperse Blue 26 [3860-63-7]	$C_{16}H_{14}N_2O_4$		298.2934
5	Disperse Blue 35 [12222-75-2]	$C_{15}H_{12}N_2O_4$		284.27
6	Disperse Blue 102 [12222-97-8]	$C_{14}H_{17}N_5O_3S$		335.38
7	Disperse Blue 106 [12223-01-7]	$C_{15}N_{16}N_6O_2S$		344.39
8	Disperse Blue 124 [61951-51-7]	$C_{16}N_{19}N_5O_4S$		377.11

Table 1. The 20 Disperse Dyes Studied, Including Chemical Abstract Services Number (in brackets), Chemical Structure, and Theoretically Calculated Exact Mass (Continued)

	Disperse dye	**Molecular formula**	**Structure**	**Mono-isotopic mass**
9	Disperse Orange 1 [2581-69-3]	$C_{18}H_{14}N_4O_2$		318.34
10	Disperse Orange 3 [730-40-5]	$C_{12}H_{10}N_4O_2$		242.2334
11	Disperse Orange 37/76 [3179-90-6]	$C_{17}H_{15}Cl_2N_5O_2$		391.0603
12	Disperse Red 1 [2872-52-8]	$C_{16}H_{18}N_4O_3$		314.3432
13	Disperse Red 11 [2872-48-2]	$C_{15}H_{12}N_2O_3$		268.2714
14	Disperse Red 17 [12222-97-8]	$C_{17}H_{20}N_4O_4$		344.3694
15	Disperse Yellow 1 [12223-01-7]	$C_{13}N_{13}N_3O_4$		275.2600
16	Disperse Yellow 3 [2832-40-8]	$C_{15}N_{15}N_3O_2$		269.3024
17	Disperse Yellow 9 [6373-73-5]	$C_{12}H_2ON_2O$		362.4250
18	Disperse Yellow 39 [12236-292]	$C_{19}H_{20}N_2O$		292.1576
19	Disperse Yellow 49 [54824-37-2]	$C_{21}H_{22}N_4O_2$		292-1576
20	Disperse Orange 11 [82-28-0]	$C_{15}H_{11}NO_2$		237.2512

Chemicals

Acetonitrile, HPLC grade	Merck (LiChrosolv, Darmstadt, Germany)
Ammonium acetate	EM Science
Dye standards mixture	Société Générale de Surveillance (SGS), Shanghai, China

LC/MS Method Details

LC Conditions

Instrument:	Agilent 1100 HPLC
Column:	ZORBAX XDB-C8, 50 mm × 2.1 mm × 1.8 µm (p/n 922700-932)
Column temp:	55 °C
Mobile phase:	A = 5-mM ammonium acetate in water B = acetonitrile
Gradient:	40% B at 0 min 60% B at 6 min 85% B at 8.5 min 95% B at 10.0 min
Flow rate:	0.8 mL/min
Injection volume:	1 µL

MS Conditions

Instrument:	Agilent LC/MSD TOF
Source:	Positive ESI
Drying gas flow:	10 L/min
Nebulizer:	40 psig
Drying gas temp:	300 °C
V_{cap}:	4000 V (negative)
Scan:	*m/z* 200–1500
References masses:	Purine (*m/z* 121.0509) and HP-0921 (*m/z* 922.0098)
Fragmentor:	120 V for $(M+H)^+$ identification and quantitation, and 320 V for fragmentation
Resolution:	10,000 @ *m/z* 922.0098

Results and Discussion

The Agilent LC/MSD TOF uses two reference masses, at both ends of the mass spectrum surrounding the mass range of interest, to perform a fine calibration on every full scan spectrum as it is saved to the data file. The electro-spray source for the LC/MSD TOF is a unique dual-spray assembly that allows the simultaneous and constant introduction of the reference mass solution.

The high mass accuracy of the LC/MSD TOF allows the user to confirm the identity as well as quantitate each compound analyzed in this work. As an example, we look closely at the Disperse Red 1 dye. Since the expected $(M+H)^+$ value is about *m/z* 315, an extracted ion chromatogram (EIC) is generated to determine the retention time (RT) for this compound. This time is determined to be 4.02 minutes. The peak in the total ion chromatogram (TIC) is shown in Figure 1.

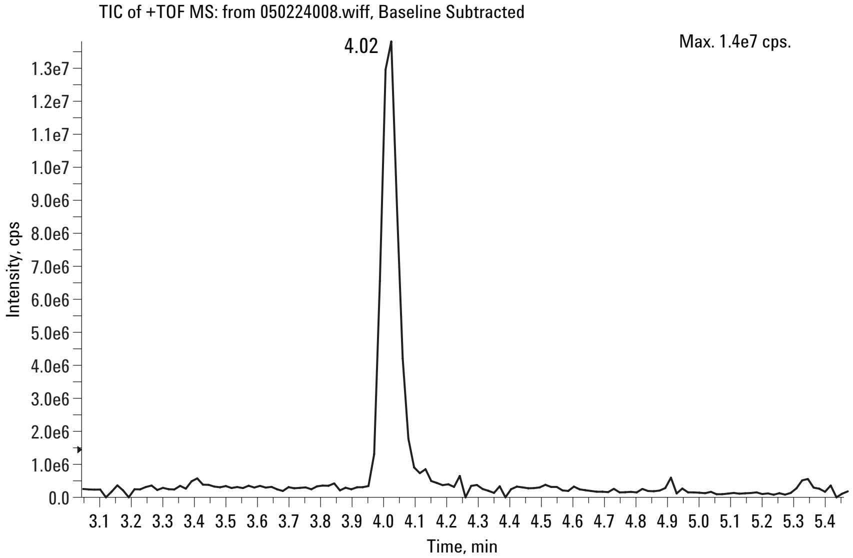


Figure 1. **TIC showing the Disperse Red 1 dye compound peak.**

By averaging the spectra under the peak, one can see the mass accuracy and resolution for the Disperse Red 1 dye. See Figure 2.

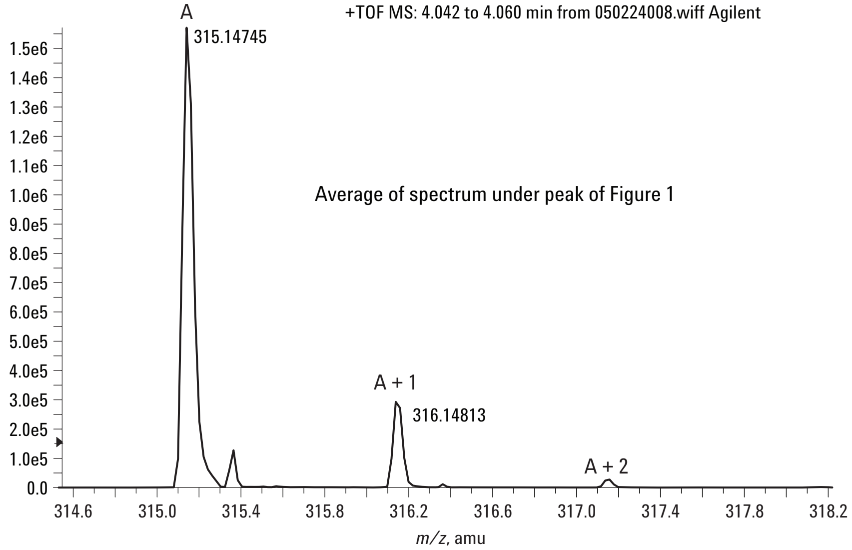
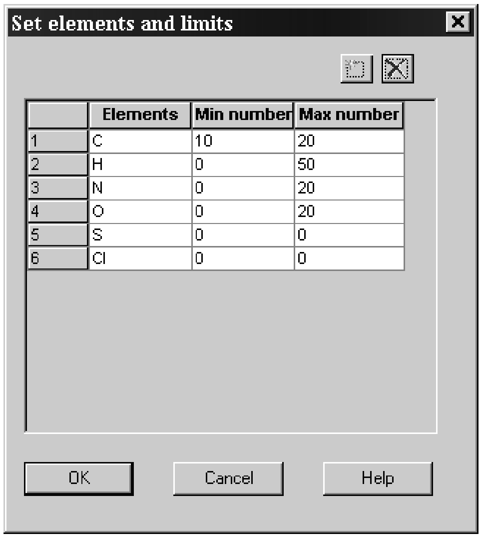


Figure 2. **Isotopic distribution of the Disperse Red 1 dye compound.**

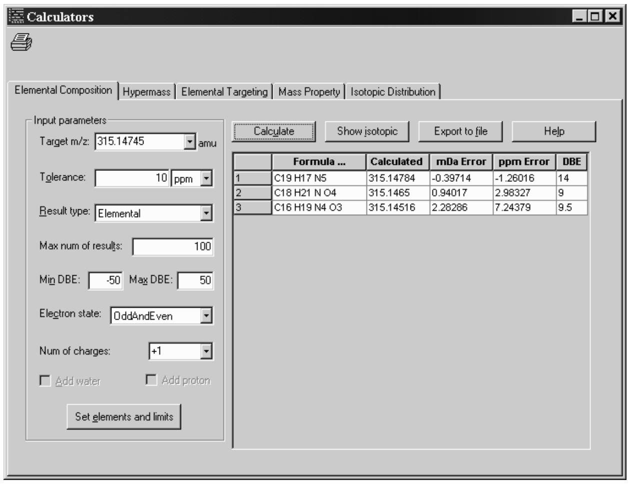
Notice the extremely high resolution separating the mono-isotopic (A), the C13 (A + 1), and the A + 2 peaks. This resolution makes for better mass accuracy and identification of the peak apex. Looking at the C13 isotope intensity at 316.14813 relative to the mono-isotopic mass at 315.14745, one can make the assumption that the amount of carbon in the empirical formula exceeds 10. Since very little A + 2 intensity is seen, we assume there is no sulfur, bromine, or chlorine present. This information is used to determine what elements should be considered for the empirical formula calculator, which is included with the TOF software. See Figure 3.



	Elements	Min number	Max number
1	C	10	20
2	H	0	50
3	N	0	20
4	O	0	20
5	S	0	0
6	Cl	0	0

Figure 3. **Elements used in empirical formula calculator.**

The calculated result is shown in Figure 4 in which three possible formulae are determined.



Target m/z: 315.14745 amu

Tolerance: 10 ppm

Result type: Elemental

Max num of results: 100

Min DBE: -50 Max DBE: 50

Electron state: OddAndEven

Num of charges: +1

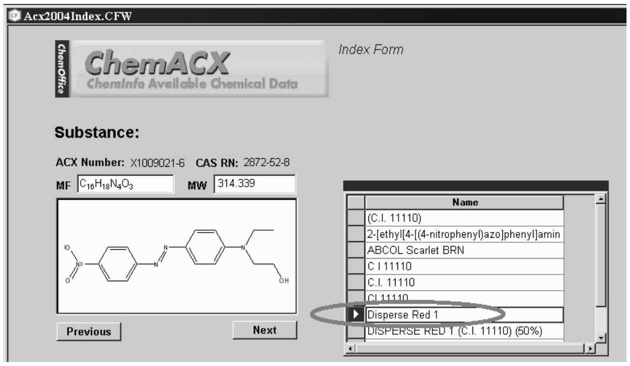
☐ Add water ☐ Add proton

	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C19 H17 N5	315.14784	-0.39714	-1.26016	14
2	C18 H21 N O4	315.1465	0.94017	2.98327	9
3	C16 H19 N4 O3	315.14516	2.28286	7.24379	9.5

Figure 4. **Three possible empirical formulae found.**

Two aspects of this calculation are noteworthy. First of all, the required mass accuracy needed to find the empirical formula corresponding to Disperse Red 1 is off by 7.24379 ppm, which is more than the system specification of 3 ppm. Either the reference masses were not intense enough for use in the fine calibration, or an isobaric interference with the Disperse Red 1 compound has skewed the mass assignment for $(M+H)^+$.

In this case, neither possibility matters because the only empirical formula that matches an entry in the ChemACX database (77,000 compounds) is $C_{16}H_{18}N_4O_3$, which is Disperse Red 1. See Figure 5 and note that the empirical formula corresponding to the mass in the spectrum corresponds to a protonated molecule, whereas the chemical formula used for the database search is for a neutral molecule, having one less H.



Substance:

ACX Number: X1009021-6 CAS RN: 2872-52-8

MF $C_{16}H_{18}N_4O_3$ MW 314.339

	Name
	(C.I. 11110)
	2-[ethyl[4-[(4-nitrophenyl)azo]phenyl]amin
	ABCOL Scarlet BRN
	C I 11110
	C.I. 11110
	CI 11110
▶	Disperse Red 1
	DISPERSE RED 1 (C.I. 11110) (50%)

Figure 5. **Database match for $C_{16}H_{18}N_4O_3$.**

The second aspect to consider in the empirical search algorithm is the mass of an electron. The LC/MSD TOF instrument has the mass accuracy necessary to determine the mass of an electron. Therefore, the calculator program needs to be told what the charge state is. For this analysis, the charge state, or 'Num of charges:' is +1.

Although the $(M+H)^+$ is identified as Disperse Red 1 using mass accuracy, a further degree of confirmation can be obtained by examining its fragment ions and whether or not they make sense structurally. Running the chromatogram again, but at a higher fragmentor voltage of 320 V, a fragment ion appears at *m/z* 255, which makes sense if the precursor ion structure loses CH_2OH, CH_3, and CH_2. See Figures 6 and 7.

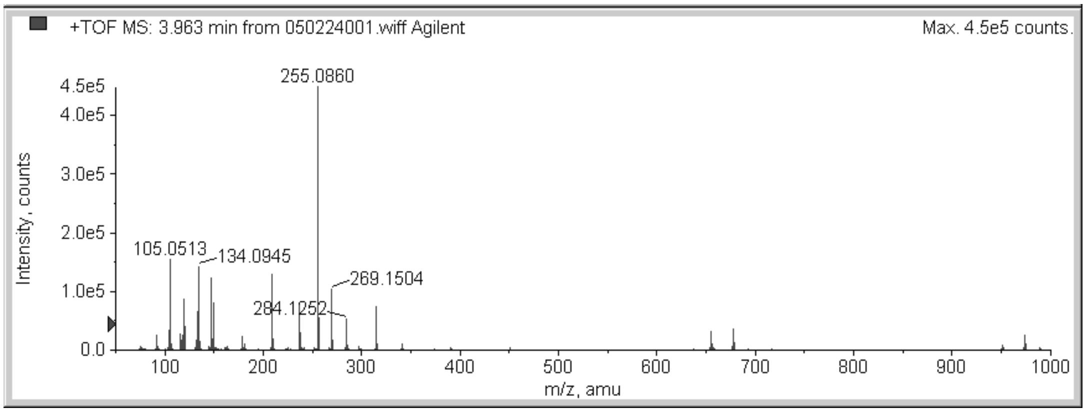


Figure 6. Spectrum of Disperse Red 1 with fragmentor = 320 V.

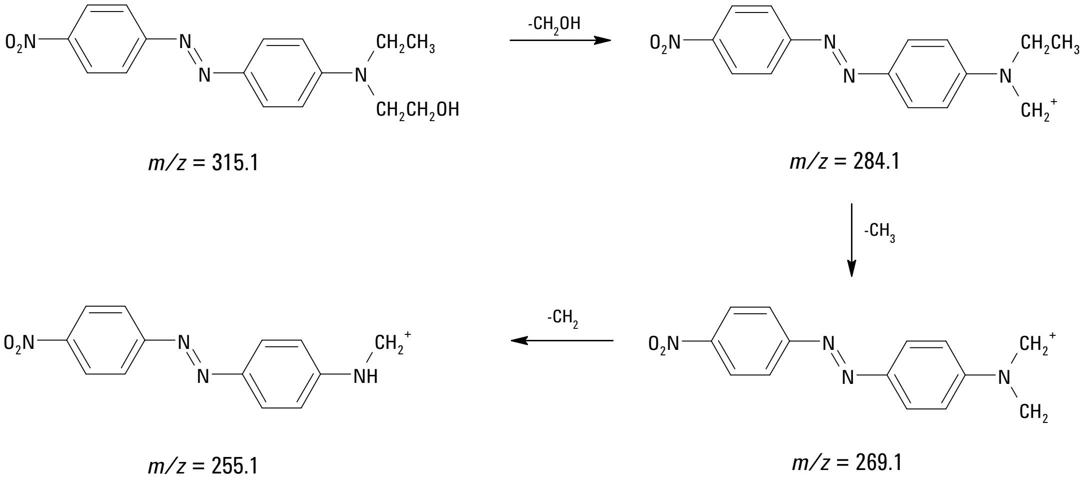
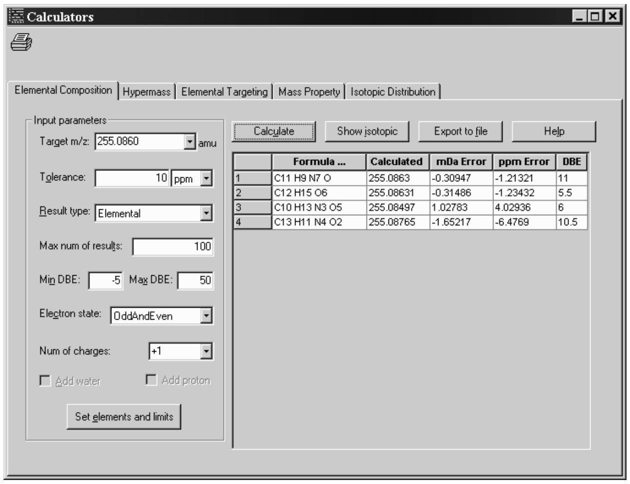


Figure 7. Possible fragmentation pathway for Disperse Red 1.

In addition to examining the fragmentation pathway, we can also use the empirical formulae calculator for the accurate mass fragment ion mass of 255.0860. See Figure 8.



Target m/z: 255.0860 amu

Tolerance: 10 ppm

Result type: Elemental

Max num of results: 100

Min DBE: -5 Max DBE: 50

Electron state: OddAndEven

Num of charges: +1

☐ Add water ☐ Add proton

	Formula ...	Calculated	mDa Error	ppm Error	DBE
1	C11 H9 N7 O	255.0863	-0.30947	-1.21321	11
2	C12 H15 O6	255.08631	-0.31486	-1.23432	5.5
3	C10 H13 N3 O5	255.08497	1.02783	4.02936	6
4	C13 H11 N4 O2	255.08765	-1.65217	-6.4769	10.5

Figure 8. Possible empirical formulae for the *m/z* 255 fragment ion.

Of the four possible results, two can be discarded because the number of nitrogen atoms is odd while the mass is odd, which is not likely for even electron fragmentation. This leaves $C_{12}H_{15}O_6$ and $C_{13}H_{11}N_4O_2$ as possible formulae for the fragment ion. However, the precursor ion only had three oxygen atoms, so the correct formula must be $C_{13}H_{11}N_4O_2$, which corresponds to loss of C_3H_8O, or CH_2OH, CH_3, and CH_2.

Once the compounds are identified and the RTs known, quantitation is performed by taking advantage of the fact that instrument mass accuracy allows the user to narrow the extracted ion current width and eliminate much of the background chemical noise. Whereas a single quadrupole instrument typically uses a mass width of 1 amu for operation in its most sensitive mode of selected ion monitoring. With the high resolution of the LC/MSD TOF in full scan mode we can quantitate using EICs of less than 1 amu. See Figure 9.

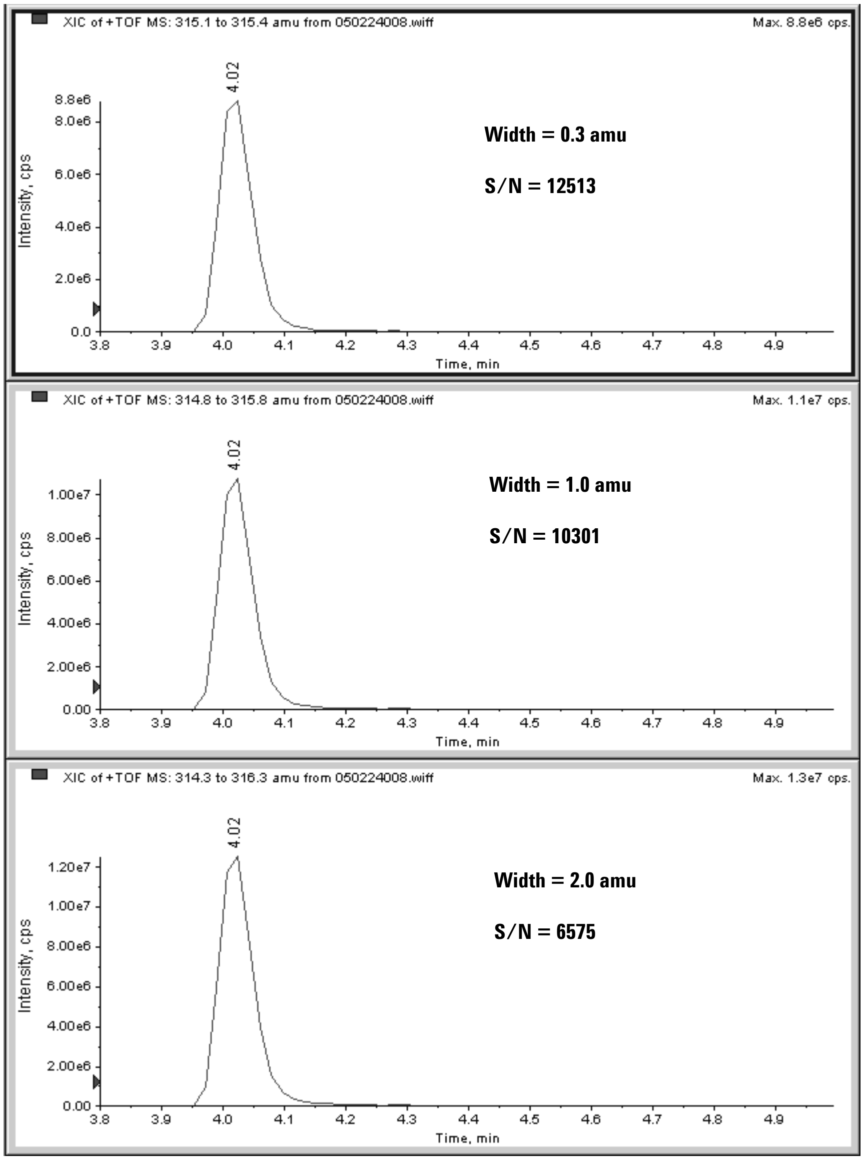


Figure 9. High mass accuracy allows for narrower EICs resulting in higher sensitivity or signal-to-noise (S/N).

As an example, linearity and limit of detection (LOD) using EICs of 0.5 amu widths for Disperse Red 1 are shown in Figure 10A and 10 B, respectively. Note that the RT shift from 4.02 min to 3.56 min was only the result of running a newer column.

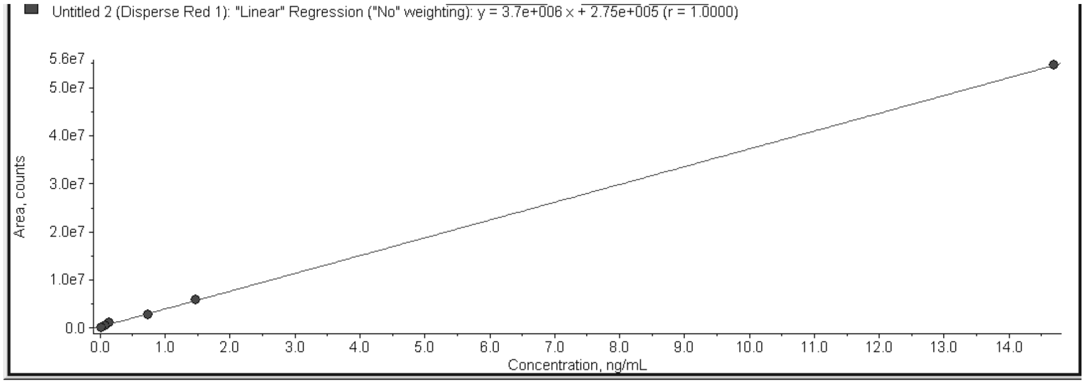


Figure 10A. Calibration result for Disperse Red 1.

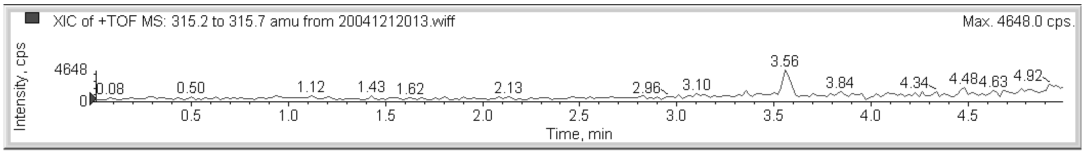


Figure 10B. EIC sensitivity for Disperse Red 1 (3.56 min), using 0.5 amu widths.

The LOD for each studied dye is given in Table 2.

Table 2. Linearity and LOD for Each Disperse Dye Analyzed Using an EIC of 0.5 amu Width

	Disperse dye	R^2	LOD (ng on-column)
1	Disperse Blue 1	0.9976	0.33
2	Disperse Blue 3	0.9992	0.42
3	Disperse Blue 7	0.9982	1.34
4	Disperse Blue 26	0.9984	0.87
5	Disperse Blue 35	0.9875	1.12
6	Disperse Blue 102	0.9921	1.22
7	Disperse Blue 106	0.9997	1.02
8	Disperse Blue 124	0.9967	1.01
9	Disperse Orange 1	0.9982	0.46
10	Disperse Orange 3	0.9983	0.08
11	Disperse Orange 37/76	0.9976	0.36
12	Disperse Red 1	0.99996	0.16
13	Disperse Red 11	0.9982	0.26
14	Disperse Red 17	0.9986	0.25
15	Disperse Yellow 1	0.9932	1.18
16	Disperse Yellow 3	0.9992	0.28
17	Disperse Yellow 9	0.9995	0.52
18	Disperse Yellow 49	0.9999	0.44
19	Disperse Yellow 39	0.9990	2.01
20	Disperse Orange 11	0.9987	0.17

Conclusions

Accurate mass measurement greatly increases the confidence of identification because it inherently limits the possible number of candidate compounds. The better the precision and accuracy of the mass measurement, the fewer compounds are theoretically possible. This is particularly useful when needing to analyze samples from a variety of sources, each with their own potential interferences such as those encountered with dye residues.

This application note demonstrated the utility of the LC/MSD TOF for the determination of low-level dyes. The LC/MSD TOF provides accurate mass determination (better than ppb) and linearity to three orders of magnitude, and thus is an excellent tool for the detection, confirmation, and quantitation of dye compounds residues.

The detection of disperse dyes has become a critical analysis in many countries. The LC/MSD TOF operated in ESI mode has the advantage that all analyses take place in full scan mode, and hence any other components may be observed. This is coupled with a sensitivity that far exceeds UV detection, and the results were shown in Table 2. Additional confirmatory information and selectivity, provided through the determination of the accurate mass, provides a very powerful technique for the detection, identification, and quantitation of dye compounds.

The high-mass resolution and accuracy capability allows for a reduction of chemical noise, thereby improving quantitation and confirmation.

LC/MSD TOF appears to be the best analytical solution because identification of unknowns could be a consideration for future regulations.

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