

ASMS 2016 TP375

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Introduction

Synthetic dyes such as azodyes and disperse dyes are widely used in the manufacture of various consumer products such as textiles, leather, toys and plastics. Many of these dyes are allergenic that may cause contact dermatitis and others are carcinogenic. Many synthetic dyes using in textiles and apparels are listed in the RSL [1] for protection of the consumers. Legislations such as EU 2002/371/EC and OEKO-TEX Standard 100 were

introduced to ban the use of carcinogenic dyes and restrict the amount of allergenic dyes allowed in textile making and consumer products. Various analysis methods using HPLC, TLC and LC/MS/MS [2] are reported. We describe a fast and high sensitivity LC/MS/MS method for screening and quantitation of 47 synthetic dyes including 23 azodyes, 21 disperse dyes and 3 basic dyes in textile samples.

Experimental

Forty-seven dye compounds were obtained from Sigma Aldrich, Dr. Ehrenstorfer, Merck Schuchardt, Fischer Scientfic, Accustandard, Institute of Leather Industry, Supelco, Fluka and Tokyo Chemical Industry. These compounds were dissolved in MeOH at 100 µg/mL as stock solutions which were used to prepare mixed standards calibrant series and spiked samples. For sample preparation, 1 gram of clothing sample was extracted using 20 mL of MeOH under sonicate at 50 °C for 30 minutes, followed by 10 minutes of centrifugation at

10,000 rpm. The supernatant was filtered with 0.22 μ m PTFE filter. It was then evaporated and reconstituted with equal volume of 95:5 water/methanol diluent before analysis. An LCMS-8040 triple quadrupole UFMS system coupled with a Nexera UHPLC was used in this study. A Phenomenex, Kinetex UHPLC column (100 x 2.1 mm, 1.7 μ m) was adopted and a gradient elution program was used for separation of the forty-seven compounds. The detailed conditions are compiled into Table 1.

Table 1: Analytical conditions of forty-seven dye compounds on LCMS-8040

Column : Kinetex C18 100A (100 x 2.1mm, 1.7μm) Flow Rate : 0.3 mL/min Mobile Phase : A: H₂O with 0.1% formic acid B: ACN with 0.1% formic acid : 40°C Oven Temp. Injection vol. Elution Mode : Gradient elution, B%: 5% (0 .01 to 2 min) \rightarrow 95% (12min) \rightarrow 100% (12.01 to 17.50min) \rightarrow 5% (17.51 to 20min) Interface : ESI MS mode : Positive & negative Block Temp. : 400°C DL Temp. : 250°C CID Gas : Ar (230kPa) Nebulizing Gas Flow : N₂, 3 L/min Drying Gas Flow : N₂, 15 L/min



Results and Discussion

Establishment of MRM method for detection and quantitation of 47 dye compounds

The MRM auto-optimisation of the 47 dye compounds was carried out using Shimadzu workstation, LabSolutions. Two MRM transitions were produced for each compound used as quantifier and confirmation ions (Table 2). A clothing sample free from the 47 dyes was used as a blank matrix used in preparing post-spiked calibrants for calibration curves construction. Every post-spiked calibrant was injected thrice to obtain the average area for reliable results. Calibration curves with good linearity (r²>0.993) were obtained for all 47 dye

compounds. The LOQ ranges from 0.06 – 4.09 ng/mL while the LOD ranges from 0.02 – 1.35 ng/mL. The repeatability of the method was evaluated using post-spiked calibrants at two concentrations, 10 ng/mL and 50 ng/mL. The %RSD (n=6) at concentration 10 ng/mL ranges from 1.2 % to 16.3 % while the %RSD (n=6) at concentration 50 ng/mL ranges from 1.1 % to 12.9 %. The linearity, LOD, LOQ and %RSD results are tabulated in Table 3.

Matrix effect and recovery of the method

The performance of the method in terms of matrix effect and recovery includes was evaluated for the 47 dyes studied too. The matrix effect and recoveries were performed at two concentrations, 10 ng/mL and 50 ng/mL. Most of the matrix effect ranges from 63.0 – 120.9 %. Four dyes, i.e., disperse red 17, disperse blue 124, disperse blue 35, and disperse yellow 49 exhibit strong matrix effect at both concentrations (ME: 31.0 %~50.9 %). While, disperse orange and disperse orange 37 exhibit strong matrix effect at 10 ng/mL

(ME: 42.1 %~49.5 %) but performed better at 50 ng/mL (ME: 71.3 %~ 87.7 %). On the other hand, another four dyes i.e., disperse blue 7, basic violet 3, disperse yellow 23 and disperse orange 149 exhibit significantly ion enhance-ment at 50 ng/mL (ME: 141.3 %~257.3 %) but performed better at 10 ng/mL (ME: 74.2 %~120.9 %). The recovery was determined from pre- and post-spiked samples of the 47 dyes, ranging from 81.8 % to 114.1 % at 10 ng/mL and from 84.9 % to 104.1 % at 50 ng/mL.

Screening and quantitation of the targeted dyes in textile samples

Three light colour clothing samples labelled as 0B, 0G and 0Y bought from the local stores were analysed using the method established. In sample 0G, disperse red 1 was detected and quantified to be 31 ng/g, which is within the regulatory limits. Samples 0G and

OY are free from the 47 dyes. Further studies on sample extraction conditions and validation of the method to various textile samples need to be performed.



Table 2: Names and information of targeted 47 dyes and MRM transitions on LCMS-8040

N	Name	CAS	Formula	Quantifier ion				Confirmation ion			
No.				MRM	Q1 (V)	CE (V)	Q3 (V)	MRM	Q1 (V)	CE (V)	Q3 (V)
1	2,4-toluenediamine	95-80-7	C7H10N2	123.10>106.05	-25.0	-20.0	-21.0	123.10>77.05	-25.0	-29.0	-29.0
2	Benzidine	92-87-5	C12H12N2	185.00>167.10	-30.0	-27.0	-30.0	185.00>168.10	-30.0	-20.0	-19.0
3	4,4'-oxydianiline	101-80-4	C12H12N2O	201.10>80.10	-30.0	-37.0	-16.0	201.10>108.10	-30.0	-20.0	-21.0
4	4,4'-diaminodiphenylmethane	101-77-9	C13H14N2	199.10>106.10	-30.0	-21.0	-21.0	199.10>77.10	-30.0	-50.0	-30.0
5	o-Anisidine	90-04-0	C7H9O1N1	124.00>109.05	-30.0	-25.0	-30.0	124.00>65.05	-30.0	-30.0	-30.0
6	o-toluidine	95-53-4	C7H9N1	108.10>91.10	-20.0	-21.0	-18.0	108.10>65.10	-20.0	-28.0	-26.0
7	p-Cresidine	120-71-8	C8H11O1N1	138.10>123.10	-30.0	-19.0	-24.0	138.10>77.05	-30.0	-34.0	-30.0
8	2,4'-Diaminoanisole	615-05-4	C7H10N2O	139.10>124.05	-14.0	-18.0	-25.0	139.10>107.05	-14.0	-17.0	-21.0
9	2,4-Xylidine	95-68-1	C8H11N	122.10>77.05	-30.0	-29.0	-30.0	122.10>107.05	-30.0	-20.0	-21.0
10	3,3'-dimethoxybenzidine	119-90-4	C14H16N2O2 (2HCI)	245.10>230.05	-17.0	-18.0	-25.0	245.10>187.10	-17.0	-33.0	-21.0
11	4- Chloroaniline	106-47-8	C6H6CIN	128.00>93.10	-30.0	-18.0	-18.0	128.00>75.05	-30.0	-34.0	-28.0
12	o-Tolidine	119-93-7	C14H16N2	213.10>180.05	-23.0	-36.0	-20.0	213.10>198.10	-23.0	-21.0	-23.0
13	4,4'-methylene-bis(2-methylaniline)	838-88-0	C15H18N2	227.10>120.10	-30.0	-26.0	-24.0	227.10>77.10	-30.0	-55.0	-29.0
14	2,6-Xylidine	87-62-7	C8H11N	122.10>77.05	-28.0	-27.0	-30.0	122.10>105.05	-28.0	-20.0	-21.0
15	2,4,5-Trimethylaniline	137-17-7	C9H13N1	136.10>121.10	-13.0	-20.0	-25.0	136.10>91.10	-13.0	-23.0	-19.0
16	2-Naphthylamine	91-59-8	C10H9N	144.10>127.05	-30.0	-24.0	-25.0	144.10>77.05	-30.0	-38.0	-29.0
17	4,4'-thiodianiline	139-65-1	C12H12N2S	217.10>124.05	-15.0	-23.0	-23.0	217.10>80.10	-15.0	-47.0	-30.0
18	4-Chloro-o-toluidine	95-69-2	C7H8CIN	142.10>107.10	-28.0	-18.0	-22.0	142.10>106.05	-28.0	-27.0	-21.0
19	Basic Red 9	569-61-9	C19H17N3.HCl	288.20>195.10	-30.0	-32.0	-20.0	288.20>167.10	-30.0	-54.0	-30.0
20	4-Aminobiphenyl	92-67-1	C12H11N	170.10>152.05	-30.0	-30.0	-30.0	170.10>153.10	-30.0	-23.0	-30.0
21	Basic Violet 14	632-99-5	C20H20CIN3.HCI	302.20>209.10	-30.0	-32.0	-23.0	302.20>195.10	-30.0	-34.0	-22.0
22	5-Nitro-o-toluidine	99-55-8	C7H8N2O2	153.00>107.10	-19.0	-17.0	-25.0	153.00>89.10	-19.0	-40.0	-30.0
23	Disperse Blue 7	3179-90-6	C18H18N2O6	359.10>283.05	-25.0	-34.0	-30.0	359.10>314.05	-25.0	-20.0	-23.0
24	Disperse Yellow 9	6373-73-5	C12H10N4O4	275.10>228.00	-20.0	-22.0	-25.0	275.10>258.10	-20.0	-15.0	-30.0
25	Disperse Blue 3	2475-46-9	C17H16N2O3	297.10>252.05	-21.0	-21.0	-27.0	297.10>251.10	-21.0	-25.0	-29.0
26	Disperse Red 11	2872-48-2	C15H12N2O3	269.10>226.10	-19.0	-30.0	-24.0	269.10>254.10	-19.0	-23.0	-28.0
27	Disperse Blue 102	12222-97-8	C15H19N5O4S	366.10>208.20	-27.0	-18.0	-24.0	366.10>147.15	-27.0	-33.0	-28.0
28	Disperse Red 17	3179-89-3	C17H20N4O4	345.20>164.15	-25.0	-27.0	-18.0	345.20>177.20	-25.0	-32.0	-20.0
29	4-aminoazobenzene	60-09-3	C12H11N3	198.10>93.20	-30.0	-25.0	-19.0	198.10>77.10	-30.0	-21.0	-30.0
30	3,3'-dichlorobenzidine	91-94-1	C12H10Cl2N2	253.00>217.10	-18.0	-19.0	-23.0	253.00>182.05	-18.0	-30.0	-22.0
31	4,4'-methylene-bis-2-chloroaniline	101-14-4	C13H12N2Cl2	267.10>231.05	-19.0	-21.0	-25.0	267.10>140.05	-19.0	-25.0	-27.0
32	Disperse Blue 106	12223-01-7	C14H17N5O3S	336.10>178.15	-24.0	-17.0	-20.0	336.10>147.10	-24.0	-32.0	-29.0
33	Disperse Orange 3	730-40-5	C12H10N4O2	243.10>122.05	-30.0	-17.0	-24.0	243.10>75.05	-30.0	-34.0	-28.0
34	Basic Violet 3	548-62-9	C25H30N3CI	372.30>356.20	-30.0	-41.0	-24.0	372.30>340.15	-30.0	-55.0	-23.0
35	Disperse Yellow 3	2832-40-8	C15H15N2O2	270.10>107.10	-30.0	-24.0	-21.0	270.10>108.15	-30.0	-31.0	-21.0
36	Disperse Orange 11	82-28-0	C15H11NO2	238.10>165.05	-17.0	-34.0	-30.0	238.10>167.05	-17.0	-37.0	-30.0
37	Disperse Brown 1	23355-64-8	C16H15Cl3N4O4	433.00>153.00	-12.0	-41.0	-29.0	433.00>196.95	-12.0	-32.0	-23.0
38	Disperse Red 1	2872-52-8	C16H18N4O3	315.10>134.10	-30.0	-27.0	-26.0	315.10>122.00	-30.0	-36.0	-24.0
39	Disperse Blue 35	12222-75-2	C20H14N2O5	285.10>270.05	-20.0	-25.0	-29.0	285.10>196.00	-20.0	-46.0	-19.0
40	Disperse Yellow 1	119-15-3	C12H9N3O5	274.20>227.20	19.0	27.0	30.0	274.20>243.05	19.0	20.0	24.0
41	Disperse Yellow 49 (Leather)	54824 - 37-2	C22H22N4O2	375.20>238.20	-27.0	-15.0	-28.0	375.20>208.15	-27.0	-39.0	-24.0
42	Disperse Blue 124	61951-51-7	C16H19N5O4S	378.10>220.15	-14.0	-17.0	-26.0	378.10>87.10	-14.0	-30.0	-17.0
43	Disperse Blue 26	3860-63-7	C16H14N2O4	299.10>284.05	-21.0	-22.0	-20.0	299.10>267.05	-21.0	-30.0	-30.0
44	Disperse Orange 37	13301-61-6	C17H15Cl2N2O5	392.10>133.05	-29.0	-38.0	-25.0	392.10>351.00	-29.0	-22.0	-26.0
45	Disperse Yellow 23	6250-23-3	C18H14N4O	303.10>77.00	-22.0	-35.0	-30.0	303.10>105.05	-22.0	-21.0	-22.0
46	Disperse Orange 1	2581-69-3	C18H14N4O2	319.10>122.05	-30.0	-23.0	-23.0	319.10>169.10	-30.0	-24.0	-19.0
47	Disperse Orange 149	85136-74-9	C25H26N6O3	457.30>121.15	16.0	49.0	19.0	457.30>149.05	16.0	43.0	24.0



Table 3: Calibration and performance of MRM method for 47 dye compounds on LCMS-8040

		DT ())					%RSD (n=6)		
No.	Compound	RT (min)	Range (ppb)	Linearity	LOQ	LOD	10 ppb	50 ppb	
1	2,4-toluenediamine	1.148	1 - 1000	0.9992	0.68	0.22	1.92	3.73	
2	Benzidine	1.506	1 - 1000	0.9960	0.52	0.17	4.89	3.88	
3	4,4'-oxydianiline	1.268	0.5 - 200	0.9944	0.26	0.09	4.47	4.12	
4	4,4'-diaminodiphenylmethane	1.583	0.5 - 1000	0.9992	0.35	0.12	1.28	2.31	
5	o-Anisidine	1.733	0.2 - 200	0.9993	0.15	0.05	4.90	2.81	
6	o-toluidine	2.049	0.2 - 200	0.9998	0.16	0.05	2.67	1.58	
7	p-Cresidine	3.975	0.1 - 200	0.9992	0.06	0.02	3.08	1.36	
8	2,4'-Diaminoanisole	3.975	1 - 200	0.9995	0.58	0.19	6.72	2.61	
9	2,4-Xylidine	3.933	1 - 200	0.9994	0.73	0.24	1.65	2.33	
10	3,3'-dimethoxybenzidine	4.514	0.2 - 200	0.9951	0.20	0.06	2.01	1.12	
11	4- Chloroaniline	4.094	1 - 200	0.9997	0.78	0.26	3.75	1.93	
12	o-Tolidine	4.395	0.5 - 200	0.9992	0.28	0.10	3.64	1.83	
13	4,4'-methylene-bis(2-methylaniline)	4.484	0.5 - 200	0.9993	0.21	0.07	2.62	2.77	
14	2,6-Xylidine	4.991	1 - 200	0.9995	0.85	0.28	3.21	3.23	
15	2,4,5-Trimethylaniline	5.001	0.5 - 200	0.9995	0.29	0.10	3.22	1.30	
16	2-Naphthylamine	5.472	0.2 - 500	0.9994	0.10	0.03	3.54	1.72	
17	4,4'-thiodianiline	5.457	0.5 - 1000	0.9993	0.19	0.06	2.91	2.00	
18	4-Chloro-o-toluidine	6.282	0.2 - 500	0.9997	0.15	0.05	1.99	2.08	
19	Basic Red 9	6.139	0.05 - 100	0.9931	0.02	0.01	1.22	1.93	
20	4-Aminobiphenyl	6.612	0.5 - 1000	0.9996	0.45	0.15	1.81	2.45	
21	Basic Violet 14	6.479	0.05 - 100	0.9977	0.02	0.01	2.78	1.42	
22	5-Nitro-o-toluidine	6.888	5 - 1000	0.9994	1.98	0.65	10.86	8.18	
23	Disperse Blue 7	7.459	1 - 200	0.9991	0.71	0.24	3.10	1.33	
24	Disperse Yellow 9	7.805	5 - 1000	0.9997	5.00	1.50	15.16	4.16	
25	Disperse Blue 3	7.921	0.5 - 200	0.9994	0.28	0.09	5.14	1.91	
26	Disperse Red 11	7.936	0.5 - 100	0.9967	0.20	0.06	3.04	2.32	
27	Disperse Blue 102	8.294	2 - 200	0.9991	0.97	0.32	16.32	7.61	
28	Disperse Red 17	8.489	0.5 - 200	0.9992	0.50	0.15	6.16	3.78	
29	4-aminoazobenzene	8.815	2 - 200	0.9995	1.23	0.41	10.43	3.95	
30	3,3'-dichlorobenzidine	8.789	2 - 200	0.9994	1.47	0.48	11.04	6.06	
31	4,4'-methylene-bis-2-chloroaniline	8.978	2 - 200	0.9993	2.00	0.60	4.77	4.59	
32	Disperse Blue 106	8.938	0.1 - 200	0.9994	0.04	0.01	10.34	3.56	
33	Disperse Orange 3	9.128	0.1 - 200	0.9994	0.08	0.03	5.81	2.01	
34	Basic Violet 3	9.2	0.05 - 200	0.9990	0.02	0.01	4.42	2.45	
35	Disperse Yellow 3	9.203	0.5 - 200	0.9991	0.21	0.07	3.97	2.74	
36	Disperse Orange 11	9.273	0.5 - 200	0.9992	0.34	0.11	11.59	4.69	
37	Disperse Brown 1	9.289	1 - 200	0.9993	1.00	0.33	6.24	8.33	
38	Disperse Red 1	9.571	0.2 - 100	0.9942	0.20	0.07	3.16	5.54	
39	Disperse Blue 35	9.875	5 - 1000	0.9980	1.98	0.65	10.54	5.99	
40	Disperse Yellow 1	8.438	2 - 200	0.9960	2.00	0.63	10.43	8.29	
41	Disperse Yellow 49 (Leather)	10.099	0.5 - 200	0.9996	0.26	0.09	3.63	2.40	
42	Disperse Blue 124	10.163	1 - 500	0.9981	0.78	0.26	4.70	4.74	
43	Disperse Blue 26	10.779	5 - 200	0.9986	4.09	1.35	7.08	12.85	
44	Disperse Orange 37	10.773	5 - 200	0.9970	4.05	1.34	4.31	3.35	
45	Disperse Yellow 23	11.049	0.2 - 200	0.9969	0.17	0.06	5.19	1.75	
46	Disperse Orange 1	11.195	0.2 - 200	0.9979	0.12	0.03	2.57	1.23	
47	Disperse Orange 149	12.069	1 - 200	0.9975	0.73	0.24	4.80	7.42	



Table 4: Matrix effects and recoveries of forty-seven dye compounds spiked in MeOH extract of blank textile matrix (white cloth).

		Matrix E	Effect (%)	Recovery (%)		
No.	Compound	10 ppb	50 ppb	10 ppb 50 ppb		
1	2,4-toluenediamine	100.0	99.0	101.5	101.4	
2	Benzidine	109.0	101.7	92.4	96.1	
3	4,4'-oxydianiline	96.7	100.7	102	94.6	
4	4,4'-diaminodiphenylmethane	109.1	105.7	99.3	95.6	
5	o-Anisidine	119.0	109.3	107.2	96.8	
6	o-toluidine	108.0	102.3	107.9	97.7	
7	p-Cresidine	118.6	109.4	107.0	98.9	
8	2,4'-Diaminoasole	117.7	106.3	102.6	98.8	
9	2,4-Xylidine	116.2	106.4	107.0	97.3	
10	3,3'-dimethoxybenzidine	111.1	102.6	102.5	97.8	
11	4- Chloroaniline	109.0	108	111.4	98.1	
12	o-Tolidine	113.6	108.7	101.6	95.6	
13	4,4'-methylene-o- toluidine	97.8	105.5	103.9	98.4	
14	2,6-Xylidine	109.5	104	106.6	98.9	
15	2,4,5-Trimethylaniline	115.8	109.9	105.5	98.5	
16	2-Naphthylamine	108.5	113.3	105.2	100	
17	4,4'-thiodianiline	86.7	92.6	95.0	98.9	
18	4-Chloro-o-toluidine	106.6	110	108.0	97.7	
19	Basic Red 9*	109.6	92.5	81.8	86.3	
20	4-Aminobiphenyl	97.2	100.0	102.3	98.0	
21	Basic Violet 14*	114.3	93.6	86.5	87.1	
22	5-Nitro-o-toluidine	104.0	116.4	110.1	100.3	
23	Disperse Blue 7	114.5	141.3	100.5	89.0	
24	Disperse Yellow 9	88.9	110.5	106.7	94.2	
25	Disperse Blue 3	87.8	101.4	103.5	95.3	
26	Disperse Red 11	89.8	86.9	90.4	91.4	
27	Disperse Blue 102	76.0	80.9	100.2	88.8	
28	Disperse Red 17	31.0	50.9	97.5	95.5	
29	4-aminoazobenzene	78.1	90.4	96.5	98.4	
30	3,3'-dichlorobenzidine	72.0	90.6	114.1	99.1	
31	4,4'-methylene-bis-2-chloroaniline	82.1	89.6	110.4	103.0	
32	Disperse Blue 106	82.9	88.7	94.2	95.9	
33	Disperse Orange 3	68.2	73.9	100.4	95.3	
34	Basic Violet 3	120.9	167.8	104.7	90.2	
35	Disperse Yellow 3	72.5	82.5	97.7	94.4	
36	Disperse Orange 11	82.9	89	101.3	94.9	
37	Disperse Brown 1	63.0	94.1	109.6	98.0	
38	Disperse Red 1	71.7	66.1	100.7	104.1	
39	Disperse Blue 35	34.7	41.7	104.0	93.1	
40	Disperse Yellow 1	113.2	105.5	101.3	100.1	
41	Disperse Yellow 49 (Leather)	38.3	45.6	103.5	97.8	
42	Disperse Blue 124	31.4	48.5	96.3	93.1	
43	Disperse Blue 26	77.1	105.7	99.3	89.2	
44	Disperse Orange 37	49.5	71.3	104.6	99.2	
45	Disperse Yellow 23	74.2	187	105.7	84.9	
46	Disperse Orange 1	42.1	87.7	104.6	85.7	
47	Disperse Orange 149	114.1	257.3	107.0	87.6	



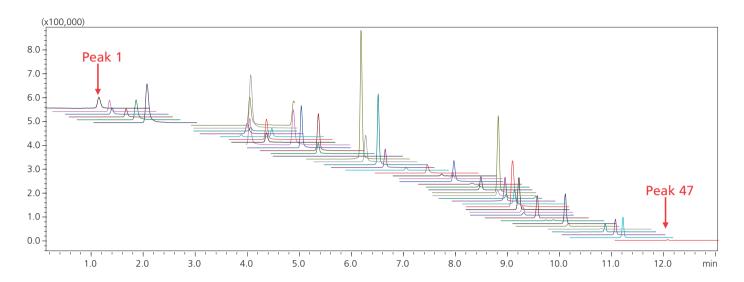


Figure 1: Scheduled MRM chromatograms of 47 dyes spiked in textile blank matrix at 20 ng/mL (ppb) each compound (5 μ L injection volume). Peak sequence refers to Table 3.

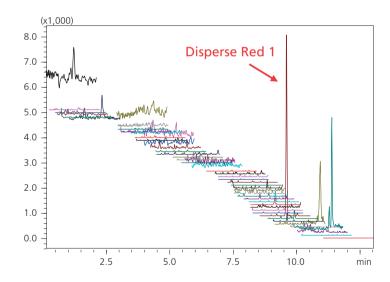


Figure 2: MRM chromatogram of sample 0G.



Conclusions

A fast and highly sensitive LC/MS/MS method has been developed on LCMS-8040 for screening and quantitation of 47 restricted or banned synthetic dyes, including 23 azodyes, 21 disperse dyes and 3 basic dyes. The total run

time of the method is less than 20 mins. The LOQs of the method for these dyes are at 0.1~4.1 ng/mL, which are fully complied with the current regulatory requirements.

References

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First Edition: June, 2016