PO-CON1736E

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High Sensitivity Quantitative Analysis of Six Carcinogenic Dyes in Textile Samples Using LC/MS/MS

ASMS 2017 TP727

Yin Ling Chew[1]; Jie Xing[1]; Felicia Xue Qi Tan[2]*;
Yi Hao Wee[3]*; Jarren Ling Yao Tuang[2]*;
Djohan Kesuma[1]; Zhaoqi Zhan[1]
[1]Shimadzu (Asia Pacific) Pte Ltd,
79 Science Park Drive #02-01/08, Singapore 118264;
[2]School of Physical & Mathematical Science,
Nanyang Technological University SPMS-CBC-02-01
Singapore 637371, Singapore;
[3]Department of Chemistry, Faculty of Science,
National University of Singapore,
21 Lower Kent Ridge Road, Singapore 119077,
*Student

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Introduction

Synthetic dyes are commonly used in manufacturing such as the textile and leather industries. Some of these chemical dyes are known to be carcinogenic, allergenic and/or hazards. Due to potential health risks, legislations such as European Union (EU) 2002/37/EC and Oeko-Tex Standard 100 were introduced to restrict the use of some synthetic dyes in textiles. Analysis methods for detection and quantitation of synthetic dyes using HPLC and LCMS have been reported [1-3]. We reported previously [4] a MRM-based LC/MS/MS method for quantitative screening of 47 commonly-used synthetic dyes in textiles, including azodyes, disperse and basic dyes. In this study, a new LC/MS/MS method was developed for quantitative analysis of six specific carcinogenic dyes in textiles, which could not be analysed steadily by the previous method established for 47 synthetic dyes on LCMS-8040 [4]. These six dyes are Acid Red 26, Direct Red 28, Direct Black 38, Direct Blue 6, Direct Brown 95 and Basic Blue 26. The first five compounds are aromatic azodyes with two or more $-SO_3$-Na^+ groups. The Basic Blue 26 is a chloride of amine with three aromatic amine groups in the structure. Both the large molecular sizes and multi-ionic groups of the six dyes make them difficult to be analysed with a single LC/MS/MS method. We describe here a new MRM method optimized for enabling simultaneous analysis of the six dyes in textiles with high sensitivity.

Experimental

The standards of the six dyes were obtained as powders from Dr. Ehrenstorfer (Germany), Tokyo Chemical Industry (Japan) and Sigma-Aldrich (USA). The solid standards were dissolved in methanol at a concentration of 100 µg/mL with the stock solutions kept at -20°C. The stock solutions were used to prepare the calibrant series and spiked samples. A Shimadzu LCMS-8045 triple quadrupole LC/MS/MS system was used to develop the MRM method for quantitative analysis of the dyes. A Phenomenex Gemini C18 column (150 x 2.0 mm, 3 µm) was used for the separation of the six dyes using a 10 minutes' gradient elution program. Table 1 shows the analytical conditions on LCMS-8045. Textile samples were obtained from local clothing shop. The clothing sample was cut to small pieces.1.0 g of the clothing sample was extracted in 20 mL of MeOH with 0.25% triethylamine under sonication at 60oC for 1 hour. The MeOH extract with washing solvent (MeOH, 5 mL) was transferred to a glass tube. The extract was blown dry using N_2 gas and reconstituted with mixed solvent (water:MeOH = 95:5). It was centrifuged at 10,000 rpm for 10 minutes to sediment the cloth fibres. The clear solution obtained was filtered with 0.22 µm PTFE filter before injection into the LCMS-8045 system.

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Table 1: LC/MS/MS analytical conditions for six carcinogenic dyes on LCMS-8045

Column	: Gemini 3u C18 110A (150 mmL. x 2.0mm I.D., 3µm)
Flow Rate	: 0.4 mL/min
Mobile Phase	: A : Water with 0.003% ammonium hydroxide B : 95:5 ACN:water with 2 mM ammonium acetate
Elution Mode	: Gradient elution, LC program 10 minutes 5%B (0.00 mins to 1.00 mins) → 80%B (4.00 min) → 95%B (4.10 mins – 7.50 mins) → 5%B (7.51 mins to 10.00 mins)
Oven Temperature	: 40 °C
Injection Volume	: 10µL
Interface	: ESI
MS Mode	: Positive and negative modes
Block Temp.	: 400 °C
DL Temp.	: 300 °C
Interface Temp.	: 400 °C
CID gas	: Ar (230 kPa)
Nebulizing Gas Flow	: Nitrogen, 3.0 L/min
Heating Gas Flow	: Zero air, 15 L/min

Results and Discussion

Optimized MRM method for six specific dyes

Under the mobile phase and gradient elution conditions shown in Table 1, the six dyes studied are eluted and detected at 1.4~8.2 mins on LCMS-8045 with ESI interface. As shown in Figure 1, the five azodye peaks are sharp and elute faster than Basic Blue 26, which appeared as a broad peak at 8.2 min and its retention was found to be sensitive to the pH of the mobile phase. The precursors formed of the compounds under this condition are either singly-charged ion or doubly-charged ions (Table 2) in negative mode except Basic Blue 26, which forms only positive ion. Acid Red 26 and Direct Black 38 also form positive ions, which are not selected to set up MRM quantitation method in this study. The precursors listed in Table 1 were subjected to MRM optimization and produced at least three MRM transitions for each compound. The details of an optimized MRM method established are shown in Table 2.

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Table 2: Information of six carcinogenic dyes and optimized MRM transitions on LCMS-8045

Name	Formula	CAS No.	Retention time (min)	Precursor & m/z value	MRM Transition	CE (V)	Ratio
Direct Blue 6	$C_{32}H_{20}N_6O_{14}S_4$. 4Na	2602-46-2	1.37	$[M-4Na+2H]^{2-}$ 421.10 (-)	249.0	24.0	100.0
					185.0	37.0	77.6
					143.1	54.0	12.9
Acid Red 26	$C_{18}H_{14}N_2O_7S_2$. 2Na	3761-53-3	3.16	$[M-2Na+H]^{1-}$ 435.05 (-)	194.0	42.0	100.0
					302.0	29.0	76.5
					222.1	33.0	38.5
Direct Red 28	$C_{32}H_{22}N_6O_6S_2$. 2Na	573-58-0	3.47	$[M-2Na]^{2-}$ 325.20 (-)	152.1	22.0	100.0
					416.1	16.0	55.0
					81.0	30.0	43.3
Direct Brown 95	$C_{31}H_{18}CuN_6O_9S$. 2Na	16071-86-6	3.56	$[M-2Na]^{2-}$ 356.60 (-)	186.0	25.0	100.0
					275.0	17.0	74.1
					314.1	20.0	51.6
Direct Black 38	$C_{34}H_{25}N_9O_7S_2$. 2Na	1937-37-7	3.74	$[M-2Na+H]^{-}$ 736.20 (-)	672.2	28.0	100.0
					644.2	32.0	44.8
					357.1	39.0	56.8
Basic Blue 26	$C_{33}H_{32}N_3$. Cl	2580-56-5	8.21	$[M-Cl]^{+}$ 470.25 (+)	349.2	-38.0	70.5
					333.2	-55.0	64.1
					454.2	-45.0	100.0

Method Performance Evaluation

A clothing sample free from the 6 carcinogenic dyes was used as a blank matrix to prepare a post-spiked calibrant series. Each post-spiked calibrant was injected thrice to obtain an average area in calibration curve construction for good reliability. Calibration curves with good linearity (>0.994) were obtained for all 6 carcinogenic dye compounds. The LOD ranges in 0.2 ~ 3.2 ng/mL while the LOQ ranges in 0.5 ~ 20 ng/mL except for Direct Black 38, which LOD and LOQ are 15 and 50 ng/mL, respectively. The linearity, LOD, LOQ and %RSD (n=6) at three concentrations for each dye except for Direct Red 28 are tabulated in Table 3. The recovery and matrix effect of the method were evaluated by using spiked samples at different concentrations. At each concentration, a duplicate set of samples was used and each duplicate was injected thrice to obtain the average area for calculations. The results of the recovery and matrix effect evaluation are tabulated in Table 4.

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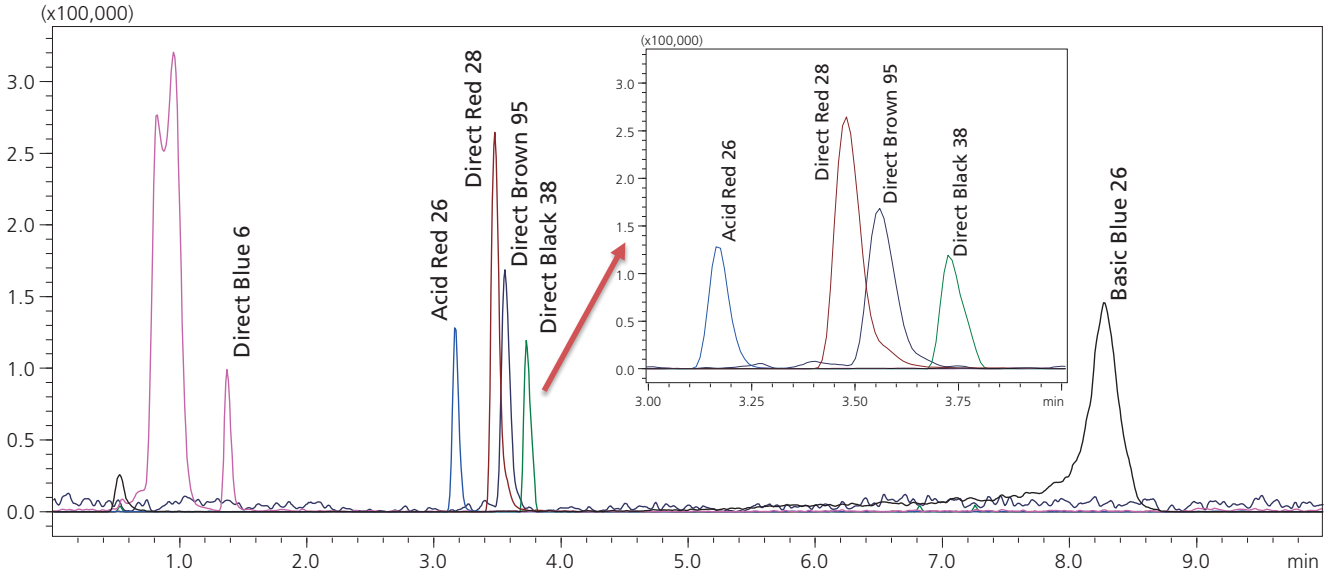

Figure 1: Total MRM chromatograms of 100 ng/mL mixed standards of 6 dyes

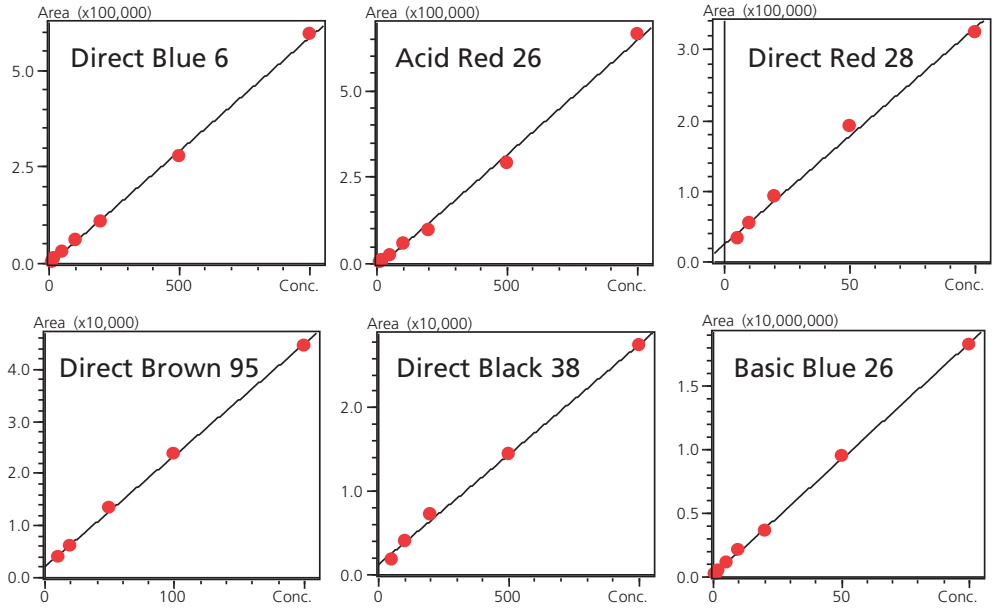

Figure 2: Calibration curves of 6 dyes (post-spiked)

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Table 3: Linearity, LOQ, LOD and repeatability results of 6 dyes spiked into clothing sample

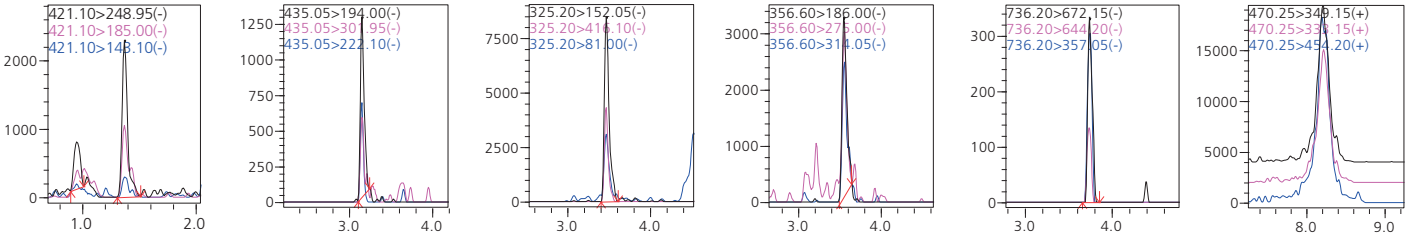
No	Compound	MRM	Range (ppb)	Linearity	LOQ (ppb)	LOD (ppb)	% RSD (n=6)		
							50 ppb	100 ppb	200 ppb
1	Direct Blue 6	421>249(-)	10 - 1000	0.9986	9.8	3.2	5.2	3.3	4.0
2	Acid Red 26	435>194(-)	10 -1000	0.9965	3.8	1.5	4.5	3.8	2.8
3	Direct Red 28	325>152(-)	5 – 100	0.9942	~5	~1.5	2,0	1.8	N.A.
4	Direct Brown 95	357>186(-)	10 - 200	0.9991	~20	~7	6.3	7.5	3.5
5	Direct Black 38	736>672(-)	50 - 1000	0.9971	~50	~15	22.2	22.0	12.2
6	Basic Blue 26	470>349(+)	0.5-100	0.9996	~0.5	~0.2	1.7	2.2	4.0

Table 4: Recovery and matrix effect studies of 6 carcinogenic dye compounds in clothing sample

No	Compound	MRM	Average Recovery (%)			Average Matrix Effect (%)		
			50 ppb	100 ppb	200 ppb	50 ppb	100 ppb	200 ppb
1	Direct Blue 6	421>249(-)	71.9	76.2	80.6	132.0	114.7	122.3
2	Acid Red 26	435>194(-)	69.9	88.1	95.9	45.7	49.6	53.8
7	Direct Red 28	325>152(-)	48.8	56.2	N.A.	48.4	54.9	N.A.
9	Direct Brown 95	357>186(-)	48.6	53.8	74.4	116.3	125.6	87.6
6	Direct Black 38	736>672(-)	78.2	87.8	125.7	242.4	153.3	103.7
8	Basic Blue 26	470>349(+)	23.6	20.0	30.3	248.5	278.2	179.5

Quantitative analysis of real clothing samples

Three real samples labelled W01, B07 and N09 bought from the local stores were analysed using the above method. All three samples are free from the 6 carcinogenic dyes. The MRM chromatograms of post-spiked textile samples at closest to the LOQ levels are displayed in Figure 3 to demonstrate detection sensitivity of the method in textile.



Direct Blue 6 (10 ppb) | Acid red 26 (10 ppb) | Direct red 28 (5 ppb) | Direct Brown 95 (50 ppb) | Direct Black 38 (50 ppb) | Basic blue 26 (1 ppb)

Figure 3: Individual MRM peaks of 6 dyes post-spiked in textile matrix at near LOQ levels

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Conclusions

A sensitive and reliable LC/MS/MS method has been developed and evaluated for analysis of six carcinogenic dyes, Acid Red 26, Direct Red 28, Direct Black 38, Direct Blue 6, Direct Brown 95 and Basic Blue 26, in textiles on LCMS-8045 system. These dyes could not be analysed steadily by the previous method which was used for quantitative screening of 47 commonly-used dyes [4] due to their large molecular size and multi ionic groups which affect the stability of LC elution and ionization. The method performance including sensitivity, linearity, repeatability, recovery and matrix effect were carried out and the results show that the method is feasible and reliable for determination of dyes in clothing samples.

References

1. Ying Zhou, Zhenxi, Du and Yun Zhang; Talanta. 127 (2014) 108 – 115
2. J. Garcia-Lavandeira, E Blanco, C. Salgado and R. Cela, Talanta, 82 (2010) 261 – 269.
3. Ding Youchao, Cao Xizhong, Wu Lina, Zhang Qian, Chinese Journal of Chromatography, Vol 26, No 5 (2008), 603 - 607
4. Yin Ling Chew, Jie Xing, Leonard Guan Seng Lim, Zhaoqi Zhan, ASMS 2016 poster – TP375

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First Edition: June, 2017

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