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Application News

No. AD-0053

LCMS-2020

Quantitative Screening of Twenty Six Aromatic Amines Originated from Azo Dyes by LC/MS Method

❑ Introduction

Azo dyes are synthetic organic colorants that contain at least one azo group (-N=N-) in the chemical structure. Azo dyes are widely used in the textile and leather industries for the outstanding color fastness and wide spectrum. However, they may undergo cleavage reaction under reductive conditions and, as a result, release aromatic amines if the azo group bonding with aromatic species. Many aromatic amines are known to be carcinogenic to humans. The European Union (EU) started new regulation on the use of azo dyes in consumer goods in Sep 2003, prohibiting the use of those azo dyes which can break down to release aromatic amines. A total of 22 aromatic amines from azo dyes had been listed in the Annex I of Directive 2002/61/EC [1, 2]. There are two analytical approaches: direct analysis of the banned azo dyes and indirect analysis of the listed aromatic amines produced from azo dyes under reductive conditions [3, 4]. The EU directives also set a limit of 30 ppm for each of the 22 aromatic amines in the final articles or parts when analyzed according to the official methods [2]. This application news reports a quantitative screening method targeted for 26 aromatic amines using simultaneous SIM/scan modes on Shimadzu ultra fast LCMS-2020. However, sample pretreatment to convert azo dyes to amines was not included.

❑ Experimental

A simultaneous SIM/Scan LC/MS method was set up for 26 aromatic amines (see Table 2). The details of the analytical conditions are shown in Table 1. The SIM mode of the method was used for sensitive detection and quantitation of the amines, while the scan mode was used as confirmation to reduce false positive result. A mixed standard sample of 26 amines was prepared in pure water with 30 ppm each compounds. The sample was diluted to 2, 6, 10 and 15 ppm for establishment of calibration curves.

Table 1: Analytical conditions of LC/MS for analysis of 26 amines using LCMS-2020

LC conditions:	
Column	Shim-pack XR-ODSIII 150 x 2 mm
Mobile Phase	A : 10mM ammonium acetate (pH 3.6) B : Acetonitrile 0.5min 10%B ->22min 60%B->28min 98%B->30min 98%B->30.1min 10%B
Flow Rate	0.20 mL/min
Oven Temp.	40 ºC
Injection Vol	10 µL

MS model:	LCMS-2020 Single Quadrupole
Interface	ESI
MS Mode	Positive SIM/Scan
Block Temp.	200 ºC
DL Temperature	250 ºC
Nebulizing Gas Flow	Nitrogen, 1.5 L/min
Drying Gas Flow	Nitrogen, 15.0 L/min

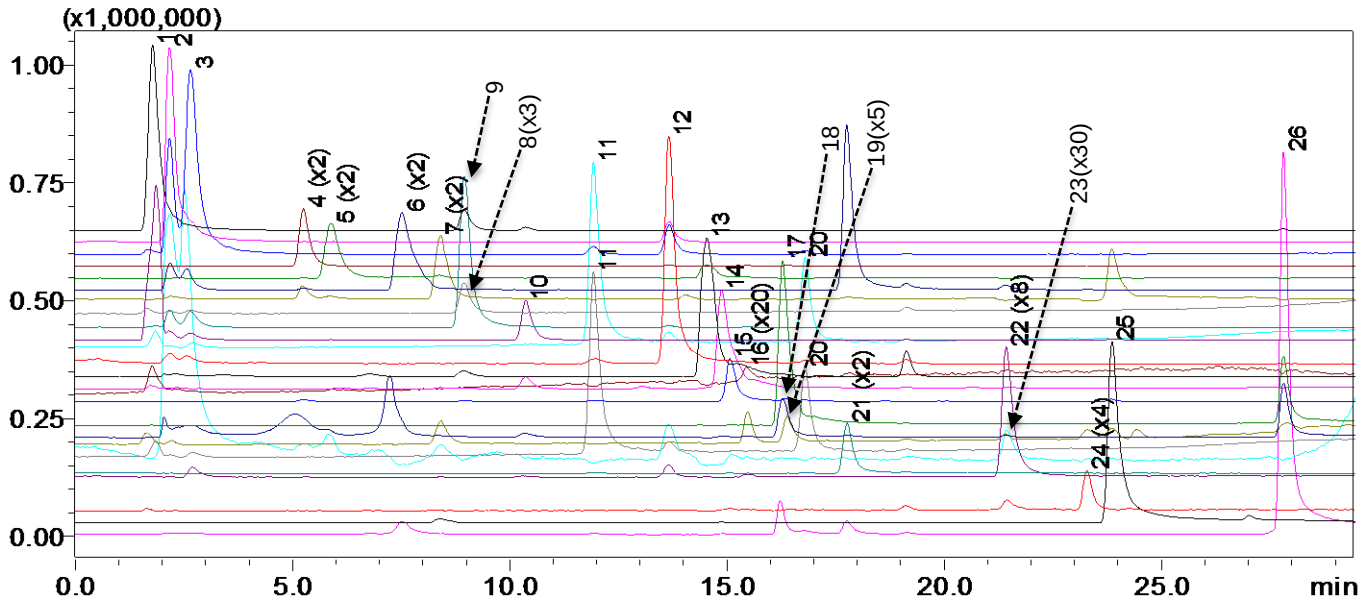

Figure 1: SIM mode MS chromatograms of a mixed standard sample of 26 amines (15 ppm each compound) on LCMS-2020

❑ Results and Discussion

As shown in Table 2, the 26 aromatic amines studied include 22 amines listed in the Directive 2002/61/EC and 1,4-Phenylene Diamine, Aniline, 2,6-Xylidine and 2,4-Xylidine. The SIM chromatograms are shown in Figure 1. The retention times ranged from 1.8 min for 1,4-Phenylene Diamine to 27.8 min for O-Aminoazotoluene. The ion form of the compounds detected was protonated ion $[M+H]^+$ in positive mode. The sensitivity of the method for every compounds was not determined purposely. However, the method could detect easily 2 ppm level for every compounds in the mixed standard sample. If a dilute factor of 20% is applied in sample pre-treatment (reduction of azo dyes and extraction), the corresponding concentration to the upper limit of 30 ppm in the analyte solution is 6 ppm. Therefore, the method can be used for screening and quantitative analysis of aromatic amines in line with the EU regulation requirement.

The basic criteria for screening analysis are the detection of the targeted mass of a compound at the expected retention time (+/-0.2 min) (Table 2) under the specified analytical conditions (Table 1). Real samples could be more complex in composition depending on the extraction method, which may cause false positive result. Mass spectrum of scan mode may help to differentiate the targeted ion and interference ion so as to avoid or reduce false positive results. Therefore, the method of data acquisition includes scan mode too. The mass spectra of the 26 aromatic amines obtained by scan mode are shown in Figure 2.

Noted that, the spectrum of 3,3'-Dimethylbenzidine (m/z 213.1) showed a strong co-occurred ion of m/z212.1 with an exactly same peak profile. The same phenomenon was also observed for 3,3'-Dimethoxylbenzidine (m/z245.1), a strong co-occurred ion of m/z244.1 accompanied. This additional "minus 1" ions for the two compounds were proven to be from the same compounds by other experiments on LCMS-IT-TOF using MS/MS and high resolution measurement. It is therefore believed that both compounds also form molecular ion M^+ under the ESI conditions. The ratio between protonated ion ($[M+H]^+$) and molecular ion (M^+) varied with mobile phase composition (details not shown in this report).

The calibration curve information of the 26 aromatic amines for a range of 2~15 ppm are shown in Table 2. Linear calibration curves between peak area (Y) and concentration (C) were obtained for every compounds, $Y = aC + 0$. The concentration of a sample is then written as $C = Y/a = R_f Y$.

Table 2: Targeted ions and retention time of 26 aromatic amines by LC/MS-2020 for screening analysis

No	Compound Name	Ret. Time (min)	$[M+H]^+$ (m/z)	CAS No (*)	Calibration		Y (6ppm)	R_f (x10^6)
					Range (ppm)	R^2	(Y=aC+0)	(1/a)
1	1,4-Phenylene diamine	1.79	109.1	106-50-3	2 - 15	0.9977	2,797,144	2.00
2	2.4-Diaminoanisole	2.17	139.1	615-05-4 (8)	2 - 15	0.9966	2,702,662	2.01
3	2,4-Tolylendiamine	2.66	123.1	95-80-7 (19)	2 - 15	0.9985	3,409,774	1.76
4	Aniline	5.25	94.1	62-53-3	2 - 15	0.9999	395,549	14.6
5	4,4-Oxydianiline	5.89	201.2	101-80-4 (16)	2 - 15	0.9901	669,749	10.7
6	Benzidine	7.52	185.2	92-87-5 (2)	2 - 15	0.9394	1,404,176	5.95
7	4,4-Diaminodiphenylmethane	8.41	199.2	101-77-9 (9)	2 - 15	0.9958	597,349	11.0
8	4-Chloro-o-toluidine	8.95	142.1	95-69-2 (3)	2 - 15	0.9972	137,168	40.3
9	o-Anisidine	8.96	124.1	90-04-0 (21)	2 - 15	0.9993	2,282,170	2.41
10	o-Toluidine	10.37	108.1	95-53-4 (18)	2 - 15	0.9999	542,864	10.5
11	2,6-Xylidine	11.93	122.1	87-62-7	2 - 15	0.9988	2,751,643	2.16
12	P-Cresidine	13.67	138.1	120-71-8 (14)	2 - 15	0.9992	3,327,459	1.71
13	3,3'-Dimethoxybenzidine	14.54	245.2	119-90-4 (11)	2 - 15	0.9849	4,004,068	2.17
14	3,3'-Dimethylbenzidine	14.88	213.2	119-93-7 (12)	2 - 15	0.9002	3,507,771	2.55
15	2,4,5-Trimethylaniline	15.07	136.2	137-17-7 (20)	2 - 15	0.9992	602,786	9.05
16	p-Chloroaniline	15.45	128.1	106-47-8 (7)	2 - 15	0.9855	5,445	970.0
17	4,4'-Methylene-bis-(2-chloroaniline)	16.28	268.1	101-14-4 (15)	2 - 15	0.9779	3,350,602	2.07
18	3,3-Dimethyl-4,4'-diaminodiphenylmethane	16.29	227.3	838-88-0 (13)	2 - 15	0.9868	823,065	9.21
19	4,4'-Thiodianiline	16.39	217.3	139-65-1 (17)	2 - 15	0.974	83,581	84.5
20	2,4-Xylidine	16.80	122.1	95-68-1	2 - 15	0.9948	1,359,861	4.77
21	2-Naphthylamine	17.77	144.1	91-59-8 (4)	2 - 15	0.9981	300,657	17.3
22	4-Aminobiphenyl	21.43	170.2	92-67-1 (1)	2 - 15	0.9847	307,563	22.2
23	2-Amino-4-nitrotoluene	21.44	153.1	99-55-8 (6)	2 - 15	0.9431	15,107	389.0
24	3,3'-Dichlorobenzidine	23.28	253.1	91-94-1 (10)	2 - 15	0.9645	834596	8.41
25	4-Aminoazobenzene	23.87	198.2	60-09-3 (22)	2 - 15	0.9998	3262362	1.84
26	O-Aminoazotoluene	27.82	226.2	97-56-3 (5)	2 - 15	0.9984	5622529	1.14

(*) Series number in EU Directive 2002/61/EC

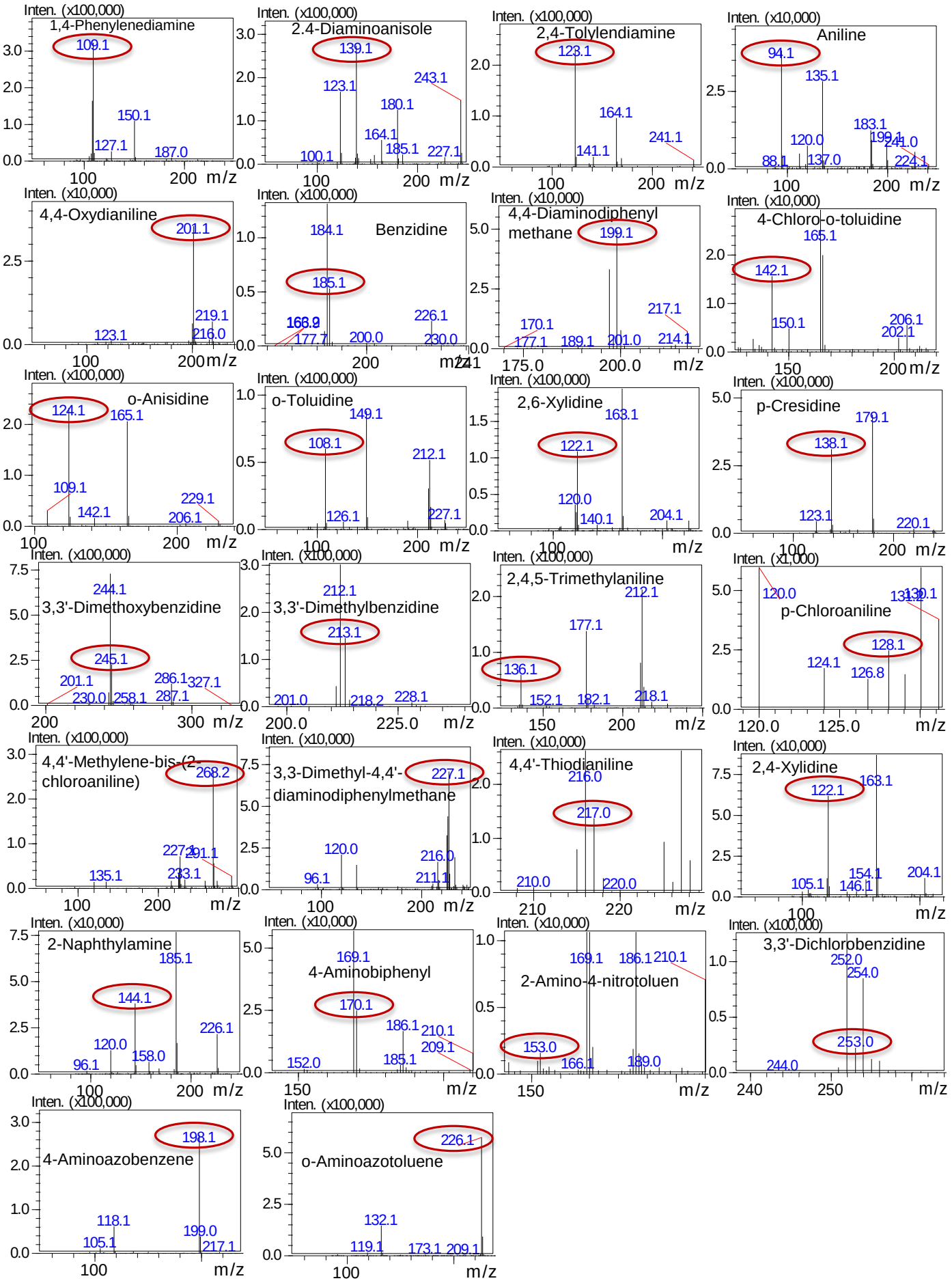

Figure 2: Scan spectra of mixed standard sample of 26 aromatic amines (15 ppm each compound) obtained by simultaneous SIM/Scan data acquisition mode on LCMS-2020.

The smaller the R_f is, the larger is the peak area of unit concentration, which is inversely proportional to the sensitivity of the method approximately. Accordingly, for example, the method is less sensitive for p-Chloroaniline (R_f = 970) and 2-Amino-4-Nitrotoluene (R_f = 389), and very sensitive for 2,4-Tolylendiamine (R_f = 1.76), P-Cresidine (R_f = 1.71), 2-Naphthylamine (R_f = 1.73) and O-Aminoazotoluene (R_f = 1.14).

Combining the identification information (m/z, RT, scan spectrum) and the quantitative information (C = R_f Y), the method established could be used as a quantitative screening for 26 aromatic amines including the 22 amines listed in the Directive 2002/61/EC.

Quantitative screening procedure

The LC/MS method established can be applied for screening and quantitation analysis of the 26 amines in real samples which are subjected to analyze for detection of banned azo dyes. The real samples must be pretreated under reduction conditions to release aromatic amines into the solution followed by extraction and concentration before injection to LC/MS for analysis. Validation of the method using actual samples and matrix must be carried out according to appropriate guidelines of industries. As a recommended quantitative screening procedure, calibration curves of the 26 amines should be established first with mixed standards under the conditions. Control samples should be prepared using an extraction matrix of a clean blank sample. Suitability test criteria must be passed. Then, the system and method are ready to run screening and quantitation analysis of real samples.

❑ Conclusions

A LC/MS screening method for detection, confirmation and quantitation of 26 aromatic amines including the 22 aromatic amines listed in the Directive 2002/61/EC) has been developed using simultaneous SIM/Scan mode on LCMS-2020. The method developed is to be used for detection and quantitation of banned azo dyes in samples. However, validation of the method for particular type of samples and sample pre-treatment (reduction of azo dyes and extraction) are required.

❑ References

1. Regulation (EC) No 1907/2006 of the European Parliament and of the Council, 18 Dec 2006
2. Directive 2002/61/EC of the European Parliament and of the Council, 19 July 2002
3. P. Sutthivaiyakit, S. Achatz, J. Lintelmann, T. Aungpradit, R. Chanwirat, S. Chumanee, A. Kettrup, Anal Bioanal Chem (2005) 381: 268–276
4. Garrigos MC, Reche F, Marin ML, Perinas K, Jimenez A, (2002) J Chromatogr A 963:427-433

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SHIMADZU (Asia Pacific) Pte. Ltd
79 Science Park Drive, #02-01 Cintech IV
Singapore Science Park I, Singapore 118264
www.shimadzu.com.sg
Tel: +65-6778 6280 Fax: +65-6778 2050

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