Screening for EU banned dyes in textiles using the Agilent 1120 Compact LC with an Agilent 6140 Single Quadrupole LC/MS system and the Analytical Studio Browser software

Increased productivity by quickly identifying samples that fail regulatory requirements

Application Note

Environmental

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Abstract

The European commission has restricted the commercialization of textiles containing certain types of azo dyes. The azo bond (-N=N-) in the dyes can undergo reductive cleavage to produce by-products such as aromatic amines, some of which are potential carcinogens. Twenty-two aromatic amines are classified by European directives as carcinogens whose concentration in textiles should not exceed 30 ppm for each amine. In this work, a literature method has been modified to include MS-compatible buffers for analysis of EU banned dyes in textiles. Analysis was performed using an Agilent 1120 Compact LC coupled to an Agilent 6140 Single Quadrupole LC/MS system and Agilent Analytical Studio Browser (ASB) software. ASB allows quick visual identification of samples that exceed regulatory requirements. Such a setup is beneficial for textile testing laboratories where large numbers of textile samples are tested for qualification.

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Introduction

The EU has listed 22 aromatic amines as potential carcinogens and maintains an acceptable upper limit of 30 ppm for each in textiles. This testing method involves the reduction of dyes in textiles with reducing agents, followed by the extraction of by-products. The by-products are matched with the list of 22 restricted amines and their concentration determined. In addition to official methods, there are other methods reported in literature that maximize the recovery. A modified literature method[1] that uses MS-compatible buffers during reduction and extraction is shown here.

An Agilent 1120 Compact LC, with the Agilent 6140 Single Quadrupole Mass Spectrometer is operated by ChemStation B.04.02.

Analytical Studio Browser (ASB) is a visual tool that allows rapid identification of samples exceeding threshold levels set by the analyst. It is an add-on software to ChemStation. The main features of ASB provide the user with the following capabilities:

- Browse very large amounts of LC/MS data very quickly.
- Assess the quality of data taken from a variety of detectors.
- Edit data and override data processing decisions made by automated systems.
- Report the data in a format that fits the particular needs of their work environment.

The methodology can be extended to analysis of other consumer samples such as toys or food products where a simple visual assessment of pass or fail is required.

Experimental

The standards of 22 restricted aromatic amines were purchased from Sigma Aldrich. Four colored polyester textile samples were purchased from local stores in India for analysis.

Twenty-two aromatic amines standard stock solution

The 22 aromatic amine standards were dissolved in acetonitrile (90:10): 25 mM ammonium acetate solution to a concentration ~3000 ppm (100% methanol also can be used). The solution was further diluted to 100-ppm solutions. The 100-ppm solutions were further diluted in 10% mobile phase B and 90% mobile phase A to 1-ppm solutions for determining the fragmentor voltage.

Textile sample

A 1.0-mL amount of freshly prepared, 1M aqueous ammonium hydroxide containing 50 mg of sodium dithionite was added to 0.1 g of shredded textile samples and heated at 80 °C for 90 min. Next, 1 mL of 100% mobile phase B was added to the textile sample and microwaved (1350 W) for 10 sec, then pipetted out. The procedure was repeated twice, using 10% mobile phase B and 90% mobile phase A as the extracting solvent. The extracted solutions were combined and 100 µL of formic acid were added to neutralize the pH. The solution was diluted to the 5-mL mark with water. An EU upper limit content of 30 ppm in 0.1 g of textile corresponds to 3 µg in 5 mL. The solution was syringe-filtered with a 0.45 µm filter before analysis.

Aqueous linearity samples

Twenty-two restricted amines were prepared to concentrations: 9 µg/5 mL, 6 µg/5 mL, 3 µg/5 mL, 1 µg/5 mL, 0.6 µg/5 mL in 5% mobile phase B.

Experimental Parameters	Details	
Column	Agilent ZORBAX Eclipse Plus C18, 150 mm × 3.0 mm, 3.5 µm p/n 959963-302; operated at 30 °C	
Mobile phase	Buffer A: Water 0.1% formic acid Buffer B: Methanol with 0.1% formic acid	
Gradient run	Run time (min): 42 min 8.8% B – 0 min 10% B – 10 min 16% B – 10.1 min 22% B – 20 min 53% B – 20.1 min 62% B – 30 min 100% B – 30.5 min 100% B – 35 min 8.8% B – 35.1 min 8.8% B – 42 min	
Flow	0.7 mL/min	
Injection volume	5 µL	
Variable wavelength detection (VWD)	254 nm	
6140 MSD parameters ESI Source: Positive mode SIM mode, peak width 0.03 min	Drying gas	13.0 L/min
	Nebulizer pressure	40 psig
	Dry gas temperature	350 °C
	Capillary voltage	4000 V
ASB parameters (B.02.00)	TIC – integration set to off at all time points BPI – 40%	

Positive control

Acid red 4 (control 1) and direct blue 15 dyes (control 2) were dissolved in methanol to dye polyester textiles. A 0.1-g sample of dyed textile was used as a positive control.

Results and discussion

Method development and analysis of a standards mixture

A mixture of 22 aromatic amines (each 1 ppm) was analyzed using VWD and MSD in series. A linear water-methanol gradient of 10% to 90% B with a 150 mm Agilent ZORBAX Eclipse Plus C18 column resolved most of the peaks well, compared to a phenyl column. In addition, methanol proved to be better than acetonitrile or a mixture of methanol and acetonitrile on a ZORBAX Eclipse Plus C18 column. A flatter gradient in combination with 30 °C column oven temperature was used to resolve overlapping peaks (9 and 10, 14 and 15). The specificity of the method was increased by operating the MSD in time-programmed SIM mode. Here, four time groups were added in data acquisition: 0 – 5 min, 5 – 10 min, 10 – 20 min and 20 min – 42 min to contain specific molecular ions in that time segment, thereby increasing the dwell time (Table 1). Figure 1 shows the MS total ion chromatogram and UV chromatogram for the standard mix (3 µg/5 mL) of 22 restricted amines. UV based detection of 22 restricted amines shows a varying response to 254 nm. The advantage of MS-based detection is increased sensitivity and selectivity compared to UV-based detection.

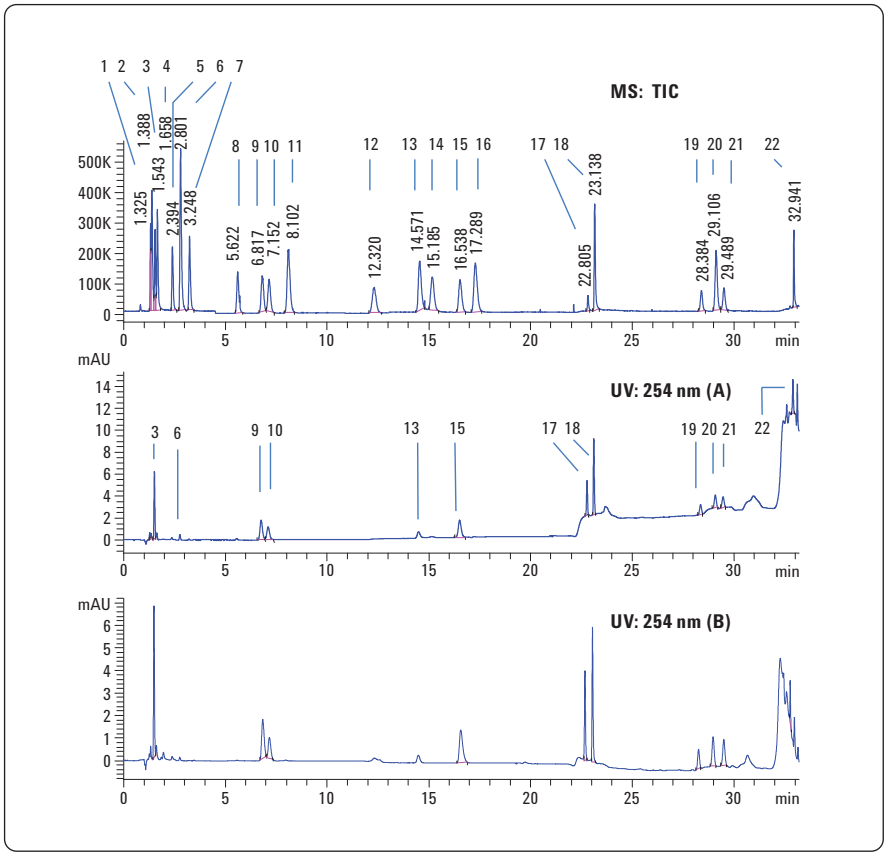

Figure 1

Total ion chromatogram (TIC) of the mixture of 22 aromatic amines operated in time-programmed SIM mode. UV detection at 254 shows varying responses to different aromatic amines. UV chromatogram (A) shows baseline shifts due to a rapidly changing gradient; UV chromatogram (B) shows the UV baseline is relatively straight when formic acid is not added in mobile phase B.

Compound name (based on time segments)	Abbreviated compound name	Molecular ion $(M+H)^+$	Fragmentor voltage (V)	Retention time (min)
Time Segment: 0 – 5 min				
4-Methoxy-*m*-phenylenediamine	1	139.1	82	1.3
2,4-Diaminotoluene	2	123.1	60	1.4
Benzidine	3	185.1	98	1.5
4,4′ -Oxydianiline	4	201.1	134	1.7
4,4′ -Diaminodiphenylmethane	5	199.1	108	2.4
o-Anisidine (2-Methoxyaniline)	6	124.1	78	2.8
o-Toluidine	7	108.1	100	3.2
Time Segment: 5 – 10 min				
4-Chloroaniline	8	128.1	110	5.6
o-Tolidine	9	213.1	112	6.8
o-Dianisidine (3,3′-Dimethoxybenzidine)	10	245.1	90	7.2
2-Methoxy-5-methylaniline	11	138.1	90	8.1
Time Segment: 10 – 20 min				
4,4′-Methylene-bis(2-methylaniline)	12	227.3	128	12.3
2-Naphthylamine	13	144.1	92	14.6
4-Chloro-2-methylaniline	14	142.1	102	15.2
4,4′-Diaminodiphenyl sulfide	15	217.1	130	16.5
2,4,5-Trimethylaniline solution	16	136.1	112	17.3
Time Segment: >20 min				
2-Methyl-5-nitroaniline	17	153.1	110	22.8
4-Aminobiphenyl	18	170.1	138	23.1
3,3′-Dichlorobenzidine solution	19	253	114	28.4
4-Aminoazobenzene	20	198.1	98	29.1
4,4′-Methylene-bis(2-chloroaniline)	21	267	144	29.5
Fast Garnet GBC base	22	226.1	94	32.9

Table 1
Fragmentor voltage and time segments used in data acquisition of EU-banned amines.

The precision of the method is demonstrated in Table 2, using six replicates of 3 µg/5 mL solution. The results show the relative standard deviation (RSD) for the retention time to be less than 0.1 min and the RSD for area response to be less than 5.6. The linearity at five concentration levels of extraction ion chromatograms show the correlation coefficient (R^2) to be greater than 0.99.

Abbreviated Compound Name	RSD of RT, n=6	RSD of Peak Area, n=6	Correlation Coefficient R^2
1	0.06	2.3	0.992
2	0.13	3.2	0.998
3	0.10	1.5	0.999
4	0.08	2.1	0.999
5	0.15	1.9	0.999
6	0.03	2.7	0.999
7	0.07	5.6	0.997
8	0.03	2.6	0.999
9	0.06	3.3	0.999
10	0.04	1.8	0.999
11	0.08	2.6	0.999
12	0.11	3.4	0.999
13	0.03	4.9	0.999
14	0.06	5.0	0.998
15	0.03	2.9	0.999
16	0.04	4.8	0.999
17	0.01	2.8	0.999
18	0.01	2.6	0.999
19	0.01	2.4	0.999
20	0.02	2.0	0.999
21	0.02	2.6	0.999
22	0.02	4.2	0.997

Table 2
The relative standard deviation (RSD) of retention time (RT) and peak area of all 22 restricted aromatic amines using six replicates of 3 µg/5 mL solutions. Correlation coefficient (R^2) value is for aqueous linearity samples.

Easy assessment of results using Analytical Studio Browser

ASB displays the results of the analysis and correlates them to the sample location in the autosampler. The integration events parameters define the peaks that are integrated. Only the integrated peaks and the percentage of base peak intensity (BPI) are used in ASB calculations to show the presence or absence of the compound in a particular sample.

Aromatic amine standards (3 µg/5 mL) were injected to determine the retention time (RT) and area. Using this database, any compound whose *m/z*, RT and area are within the acceptable range is integrated and shows as a target compound (Figure 2).

Azo dyes such as acid red 4 and direct blue 15 are known to degrade into banned aromatic amines: 2-Methoxyaniline and 3,3´-dimethoxybenzidine respectively[2]. Two polyester textiles were dyed separately using acid red 4 and direct blue 15. As shown in Figure 2, the textile sample dyed with acid red 4 produced *m/z* of 124.1 (Compound 6; 2-methoxyaniline). Similarly, the textile sample dyed with direct blue 15 produced *m/z* of 245.1 (Compound 10).

Four colored polyester textile samples were also analyzed but they did not yield any banned aromatic amines. If banned amines had been detected then the analyst could quantitate only those aromatic amines that are identified as the target. The results show that ASB provides confirmation of the presence or absence of specific banned amines; thereby eliminating the need to quantify all the 22 banned amines.

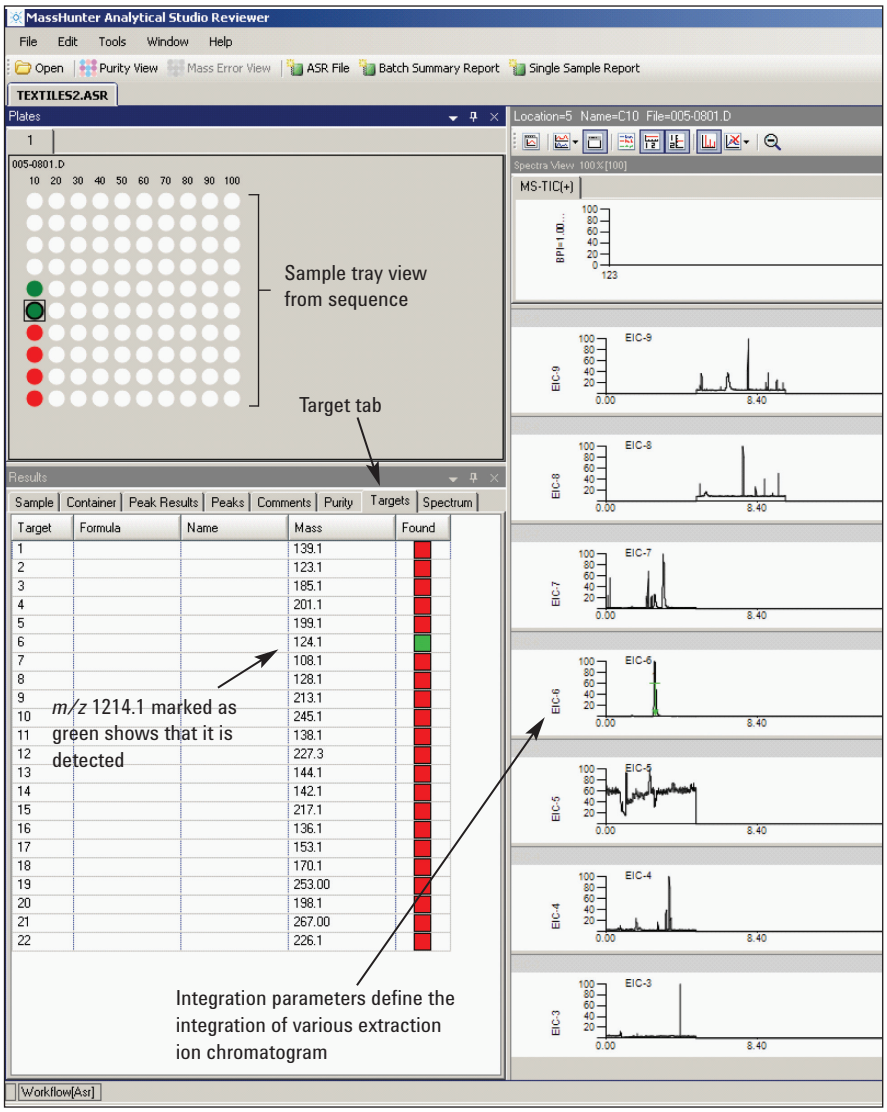

Figure 2
Screen Shot of Analytical Studio Browser showing a textile sample dyed with acid red 4 (control 1, sample position 20). ASB target list identifies 124.1 as compound detected.

Conclusion

The combination of the Agilent 6140 Single Quadrupole LC/MS system with the Analytical Studio Browser software provides a versatile tool for quick screening of textiles for compliance with regulatory standards. Quick identification of failed samples leads to reduced analysis time. Analytical Studio Browser provides visualization of samples that fail requirements.

References

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2. L. H. Ahlstrom, E. Bjorklund, L. Mathiasson, "Optimization of an analytical procedure for the determination of banned azo dyes in leather," Anal Bioanal Chem, 382: 1320-1327, 2005.

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