MATERIALS ANALYSIS

QUANTITATIVE DETERMINATION OF 26 AROMATIC AMINES DERIVED FROM BANNED AZO DYES IN TEXTILES THROUGH THE USE OF LC, TANDEM MS, AND IDENTIFICATION OF SOME STRUCTURAL ISOMERS.

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Application Note

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ABSTRACT

The determination of some aromatic amines derived from banned azo dyes is important in chemical safety control in the fashion industry. Many European and international regulations (REACH, GB and KC Mark) strictly limit their presence. The main regulations for the identification of aromatic amines derived from azo dyes define that analysis has to be conducted by at least two chromatographic methods, in order to avoid misidentification due to the presence of matrix interferences or structural isomers. In this study, an analytical method has been developed that minimises matrix clean-up and identifies the amines in an unequivocal way. This study also allows for the efficient separation of structural isomers, avoiding the need for double analysis.

INTRODUCTION:

Some azo dyes can produce, in specific conditions, some primary aromatic amines (PAAs) that are considered to be carcinogenic by the most important international authorities. For this reason, specific regulations have been introduced in order to monitor their presence in materials used in the fashion industry, such as textiles and leathers. The various regulations stipulate different groups of aromatic amines with varying concentration limits. The strictest regulation in force is the Chinese regulation GB 18401:2010 which allows for the presence of 24 aromatic amines, with a limit of 20 mg/kg for each amine.

The main research methods for the analysis of these amines use GC/MS. In many cases, detection by gas chromatography is complicated by the presence of polar analytes and by the separation of structural isomers that are not banned by international regulations. Liquid chromatography allows for a reliable, efficient method that is faster than traditional gas chromatography methods. It also allows for the efficient separation of structural isomers.

The method developed in this application note is really useful and versatile. It reliably identifies aromatic amines in many materials, avoids expensive clean-up and is able to separate the structural isomers of interest.

ANALYTICAL TECHNIQUE

Reagents and standards

Acetonitrile, water, formic acid, isomers of specific amines from Sigma-Aldrich (Milan, Italy).

Standard of 26 aromatic amines at 100 mg/L from Ultra Scientific – Analytical Standard (Bologna, Italy).

Instrumentation

HPLC separation was performed on the Agilent 1200 LC system using an Agilent Poroshell 120 SB-C18 column with detection on the Agilent 6420 Triple Quadrupole Mass Spectrometer.

Agilent 1200/6420 LC/QQQ Operating Conditions

LC Conditions		
Analytical Column	*Poroshell 120 SB-C18 3.0 x 100mm x 2.7µm*	
Column Temperature	*40°C*	
Injection Volume	*0.5 µL*	
Mobile Phase	*A = 0.05 mM Formic Acid in Water*	
	B = 0.1% Formic Acid in Acetonitrile	
Run Time	*12 minutes*	
Flow Rate	*0.6 mL/min*	
Gradient Program	*Time (minutes)*	*Gradient (% A)*
	0	*75*
	0.5	*75*
	5	*30*
	8	*5*

MS Conditions	
Acquisition Parameters	*ESI Positive Ion Mode*
Gas Temperature	*320°C*
Gas Flow	*10 L/min*
Nebuliser Pressure	*50 psi*
Capillary Voltage	*3000 V*

MRM Transitions – ESI Positive Ion Mode

Amine	CAS Number	Scan Segment	Precursor Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)	Fragmentor Voltage (V)	Collision Energy (V)
3,3'-Dimethoxybenzidine	*119-90-4*	*1*	*245.1*	*230.1*	*213*	*100*	*15*
4,4'-Methylenedi-o-toluidine	*838-88-0*	*1*	*227.3*	*195.2*	*178.3*	*100*	*15*
3,3'-Dimethylbenzidine	*119-93-7*	*1*	*213.0*	*198*	*181*	*100*	*15*
4,4'-Oxydianiline	*101-80-4*	*1*	*201.2*	*184.1*	*93.0*	*100*	*15*
4,4'-Diaminodiphenyl-methane	*101-77-9*	*1*	*199.5*	*106.1*	*89.1*	*100*	*20*
Benzidine	*92-87-5*	*1*	*184.3*	*167.5*	*93.0*	*100*	*15*
4-Methoxy-m-phenylen-ediamine	*615-05-4*	*1*	*139.1*	*124.1*	*107.3*	*100*	*15*
p-Cresidine	*120-71-8*	*1*	*138.1*	*123.4*	*106.1*	*100*	*15*
o-Anisidine	*90-04-0*	*1*	*124.1*	*109.2*	*92.2*	*100*	*15*
4-Methyl-m-phenylene-diamine	*95-80-7*	*1*	*123.3*	*106.1*	*79.2*	*100*	*15*
1,4-Phenylenediamine	*106-50-3*	*1*	*109.2*	*92.2*	*65.1*	*100*	*15*
o-Toluidine	*95-53-4*	*1*	*108.3*	*91.1*	*65.2*	*100*	*15*
Aniline	*62-53-3*	*1*	*94.1*	*77.1*	*51.1*	*100*	*15*
2,4,5-Trimethylaniline	*137-17-7*	*1*	*136.3*	*121.3*	*91.2*	*100*	*15*
2,4-Dimethylaniline	*95-68-1*	*2*	*122.1*	*105.1*	*79.2*	*100*	*15*
4,4'-Thioaniline	*139-65-1*	*2*	*217.2*	*200.1*	*183.0*	*100*	*15*
2-Naphthylamine	*91-59-8*	*2*	*144.1*	*127.4*	*117.1*	*100*	*15*

4-Chloroaniline	*106-47-8*	*2*	*128.3*	*111.2*	*93.2*	*100*	*15*
2,6-Dimethylaniline	*87-62-7*	*2*	*122.1*	*107.1*	*79.2*	*100*	*15*
5-Nitro-o-toluidine	*99-55-8*	*3*	*153.3*	*107.3*	*90.2*	*100*	*15*
p-Aminobiphenyl	*92-67-1*	*3*	*170.2*	*153.2*	*128.4*	*100*	*20*
4-Chloro-o-toluidine	*95-69-2*	*3*	*142.1*	*128.2*	*107.1*	*100*	*15*
4,4'-Methylene-bis(2-chloroaniline)	*101-14-4*	*4*	*268.2*	*232.2*	*196.1*	*100*	*20*
3,3'-Dichlorobenzidine	*91-94-1*	*4*	*254.2*	*218.1*	*183.0*	*100*	*15*
o-Aminoazotoluene	*97-56-3*	*4*	*226.1*	*121.1*	*107.1*	*100*	*15*
p-Aminoazobenzene	*60-09-3*	*4*	*198.1*	*93.1*	*77.1*	*100*	*15*

Sample Preparation

Textiles not dyed with disperse dyes were extracted using the procedure in ISO 14362-1:2012, while for leather the procedures in ISO 17234-1:2010 and 17234-2:2010 were used. The organic solutions were filtered with regenerated cellulose filters before injection.

In the case of fabrics dyed with disperse dyes, samples were initially extracted with Chlorobenzene, before continuing with the procedures listed above. In both cases, Anthracene-d10 (CAS 1719-06-8) was used as the internal standard, to give a final concentration of 10 mg/mL.

Results and Discussion

The separation of the 26 aromatic amines was achieved by optimisation of the chromatographic conditions, including the choice of chromatographic column and optimisation of the spectrometer conditions.

Separation of Isomers

The separation of structural isomers can be extremely important as regards the possibility of false positives. In many gas chromatographic separations, the elution of specific amine isomers can differ by a few seconds. This difference can be part of the statistical variability of the retention times of each isomer. For this reason many of the regulations mention simultaneous determination using more than one chromatographic method, in order to avoid misidentifications due to the presence of structural isomers or matrix interferences. The use of liquid chromatography limits these difficulties allowing for the efficient and reliable determination of each isomer. Results are shown in Table 1.

Amine isomer	CAS number	Retention time (min)
1-Naphthylamine	*134-32-7*	*3.008*
2-Naphthylamine	*91-59-8*	*2.222*
2,4-Dimethylaniline	*95-68-1*	*1.070*
2,5-Dimethylaniline	*95-78-3*	*1.275*
2,6-Dimethylaniline	*87-62-7*	*2.225*
2,3-Dimethylaniline	*87-59-2*	*1.095*
3,4-Dimethylaniline	*95-64-7*	*0.965*
3,5-Dimethylaniline	*108-69-0*	*1.145*
2-Chloroaniline	*95-51-2*	*4.430*
3-Chloroaniline	*108-42-9*	*3.495*
4-Chloroaniline	*106-47-8*	*2.095*
o-Anisidine	*90-04-0*	*0.995*
m-Anisidine	*536-90-3*	*0.960*
p-Anisidine	*104-94-9*	*0.760*
o-Toluidine	*95-53-4*	*1.045*
m-Toluidine	*108-44-1*	*0.920*
p-Toluidine	*106-49-0*	*0.860*

Table 1: *Retention times of structural isomers of some amines used in this study.*

Calibration Curves

The calibrations were produced using the external standard method with 4 calibration points at 0.1, 0.5, 1 and 5 mg/L of amines. Linear regression was used and the curves were forced through the origin. Table 2 shows the performance parameters for all the amines. All amines display optimal regression coefficients (R2) and detection limits.

Amine	Equation	R2	Regression	LOD	RSD (%)
3,3'-Dimethoxybenzidine	*y= 29934x + 553*	*0.999*	*Linear*	*1.2*	*3.1*
4,4'-Methylenedi-o-toluidine	*y= 1378x + 21*	*0.999*	*Linear*	*0.4*	*1.8*
3,3'-Dimethylbenzidine	*y= 13557x + 1027*	*0.999*	*Linear*	*1.1*	*6.5*
4,4'-Oxydianiline	*y= 2949x + 67*	*0.999*	*Linear*	*0.8*	*1.2*
4,4'-Diaminodiphenylmethane	*y= 24505x + 693*	*0.999*	*Linear*	*0.8*	*1.2*
Benzidine	*y= 1113x - 20*	*0.999*	*Linear*	*0.9*	*3.5*
4-Methoxy-m-phenylenediamine	*y= 31567x + 1226*	*0.999*	*Linear*	*1.5*	*5.2*
p-Cresidine	*y= 396223x + 8522*	*0.999*	*Linear*	*1.0*	*3.8*
o-Anisidine	*y= 401391x + 10677*	*0.999*	*Linear*	*1.3*	*0.5*
4-Methyl-m-phenylenediamine	*y= 55488x + 1330*	*0.999*	*Linear*	*3.2*	*1.4*
1,4-Phenylenediamine	*y= 43230x + 1308*	*0.999*	*Linear*	*0.6*	*1.1*
o-Toluidine	*y= 213000x + 12326*	*0.999*	*Linear*	*0.6*	*0.9*
Aniline	*y= 26451x + 17792*	*0.999*	*Linear*	*0.4*	*1.3*
2,4,5-Trimethylaniline	*y= 180357x + 2967*	*0.999*	*Linear*	*1.2*	*1.5*
2,4-Dimethylaniline	*y= 207557x + 9137*	*0.999*	*Linear*	*1.8*	*4.7*
4,4'-Thioaniline	*y= 47851x - 940*	*0.999*	*Linear*	*1.1*	*4.2*
2-Naphthylamine	*y= 118838x + 2445*	*0.999*	*Linear*	*0.3*	*1.5*
4-Chloroaniline	*y= 152241x - 1938*	*0.999*	*Linear*	*1.6*	*7.2*
2,6-Dimethylaniline	*y= 77129x - 353*	*0.999*	*Linear*	*2.1*	*3.9*
5-Nitro-o-toluidine	*y= 127200x - 51*	*0.999*	*Linear*	*17.2*	*13.5*
p-Aminobiphenyl	*y= 86449x + 440*	*0.999*	*Linear*	*4.1*	*0.4*
4-Chloro-o-toluidine	*y= 145693x - 1271*	*0.999*	*Linear*	*3.3*	*0.7*
4,4'-Methylenebis(2-chloroaniline)	*y= 3311x + 75*	*0.999*	*Linear*	*3.1*	*5.5*
3,3'-Dichlorobenzidine	*y= 5942x + 200*	*0.999*	*Linear*	*0.7*	*4.8*
o-Aminoazotoluene	*y= 154925x + 7559*	*0.999*	*Linear*	*0.7*	*1.1*
p-Aminoazobenzene	*y= 261090x + 10032*	*0.999*	*Linear*	*1.8*	*1.3*

Table 2: *Calibration curves equations, R2, LOD and % RSD for the amines used in this study.*

The percentage recovery is on average greater than 85% and is calculated using the internal standard, Anthracene-d10. Figure 1 shows a typical standard chromatogram of the 26 amines and Figure 2 shows some examples of calibration curves.

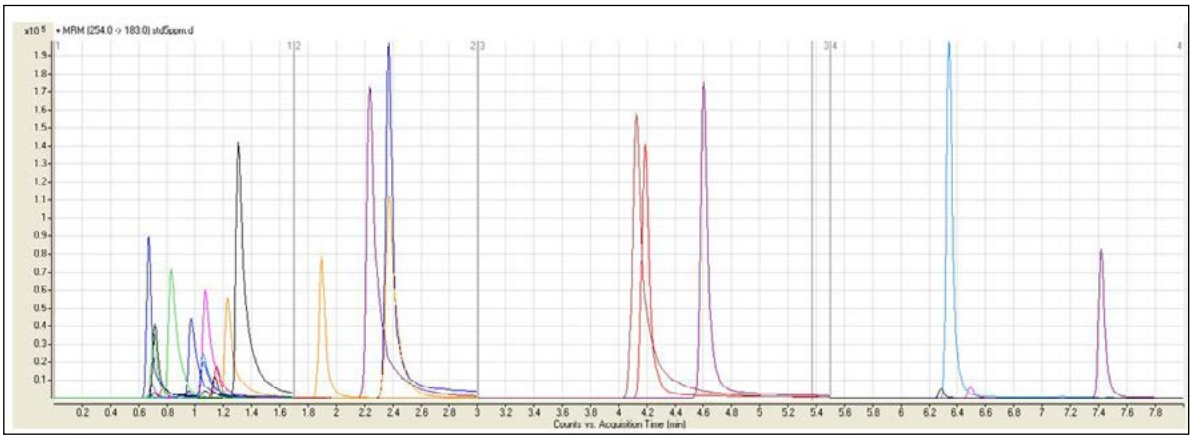

Figure 1: *Example of a standard chromatogram.*

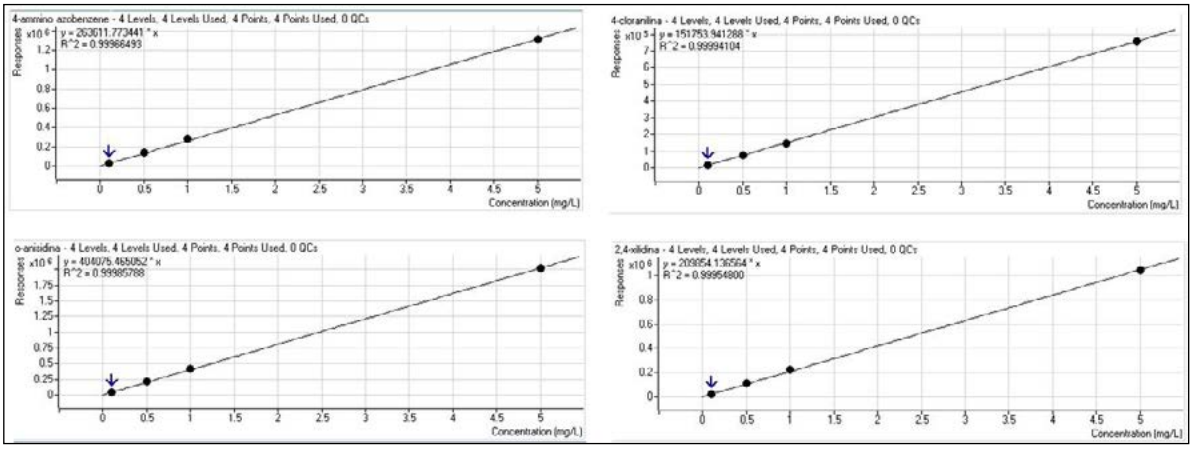

Figure 2: *Examples of amine calibration curves.*

CONCLUSIONS

This method is an extremely fast and reliable method for the determination of aromatic amines derived from azo dyes that have been banned by international regulations. Similar results were also observed using purification with sorbent packed columns, reducing sample preparation time. This method enables these aromatic amines to be screened and quantitated at the same time.

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