Analysis of EU Banned Azo Dyes in Textiles Using Liquid Chromatography Coupled With Single Quadrupole Mass Spectrometry

ASMS 2010

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Introduction

The European (EU) commission has restricted the commercialization of textiles containing certain types of 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. Figure 1 shows an example of reductive cleavage of direct blue 015 azo dye forming the aromatic amine. Twenty two aromatic amines are classified by European directives as carcinogens whose concentration in textiles may not exceed 30 ppm for each amine[1].

In this work, a LCMS method is developed to separate, identify, and quantify a mixture of twenty two aromatic amines. A literature method for chemical reduction of the textiles samples was applied[2]. A standard addition method was used for quantification wherein a standard mixture of the twenty two restricted aromatic amines were spiked into the sample at various increasing concentrations prior to reduction. This standard addition method for quantification accounts for recovery losses of the analyte during the reduction of textile samples

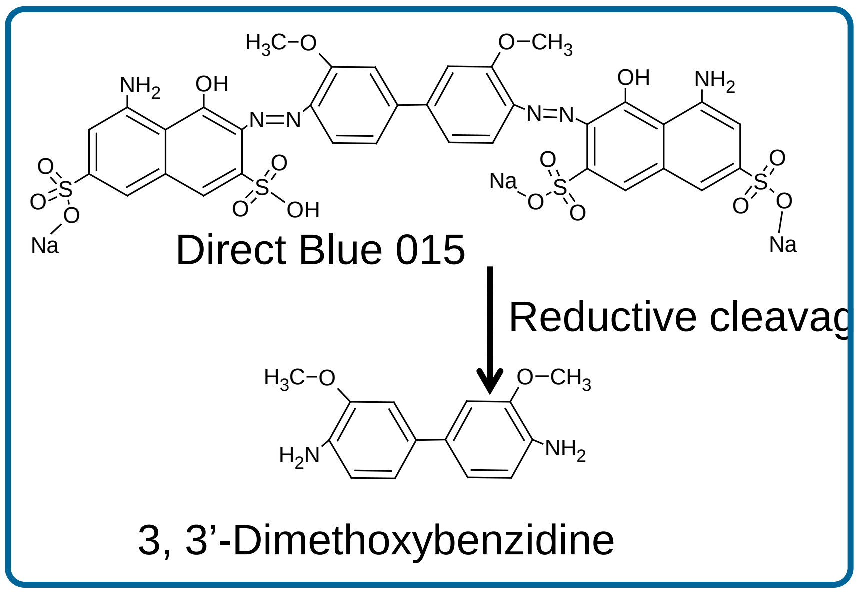

Figure 1: Schematic representation of reductive cleavage of direct blue 015 to produce 3,3'-dimethoxybenzidine (Compound 10)

Experimental

Instrument configuration

LC: Agilent 1120 Compact LC consisting of : Gradient pump with integrated degasser, Auto sampler, column oven, and Agilent Chemstation B.04.02 software.

MS: Agilent 6140 MSD: ESI Source – Positive mode (SIM mode, with time segment). Data Analyzed using MassHunter Quant/Qual analysis software incorporating the LC-SQ Chemstation Translator.

Column: Agilent Zorbax Eclipse Plus C18, 150 mm x 3.0 mm, 3.5 µm, operated at 30 C

Experimental

LC Parameters

Mobile Phase: Buffer A: Water with 0.1% formic acid
Buffer B: Methanol with 0.1% formic acid

Run time (min):42 min (post run:7 minutes)
Flow: 0.7 mL/min
Injection volume: 5 µL

Table 1;Gradient:

%B	Minutes
8.8	0
10	10
16	10.1
22	20
53	20.1
62	30
100	30.5
100	35

MS Parameters
Drying gas: 13.0 L/min
Nebulizer pressure: 40 psig
Dry Gas Temperature: 350 C
Capillary Voltage: 4000 V

Sample Preparation

Aromatic amines standard stock solution: The 22 aromatic standards were dissolved in (90:10) acetonitrile: 25 mM Ammonium acetate solution to a concentration ~3000 ppm. The solution was further diluted to 100 ppm solutions. 100 ppm solutions was further diluted in 10% mobile phase B and 90% mobile phase A to 1 ppm solution for determining the fragmentor voltage.

Linearity samples in aqueous solution:
Aqueous linearity samples included 0.02, 0.05, 0.13, 0.2, 1.21, and 1.8ppm solutions.

Textile samples: 4 colored and 1 white polyester textiles were shredded and used for the analysis.

Positive control: Acid Red 4 (Control 1) and Direct Blue 15 dyed (Control 2) were dissolved in methanol and dyed to polyester textiles. 0.1 g of respective dyed textile was used as a positive control.

Standard addition: Out of four different textile samples. Three were spiked with 50 µL of 22 aromatic amine standard mix at 0.13, 0.2, 1.21ppm concentrations respectively keeping one sample un spiked.

Sample reduction procedure: 1 mL of 1M NaOH containing 50 mM Sodium dithionite is added to 0.05 g of shredded textiles. Samples are kept at 80 C for 90 min.

Extraction procedure: 1 mL of 50 mM Ammonium acetate and 50 µL of formic acid is added to the samples, followed by 4 mL of 100% ethyl acetate. 1 mL of the supernatant is vacuum evaporated and dissolved in 1 mL of 5% mobile phase B for analysis.

An upper limit of 30 ppm in 0.05 g of textile sample would correspond to 1.5 µg in 4 mL supernatant.

Results and Discussion

Chromatographic representation of 22 aromatic amines are as shown in Figure 2. Name and abbreviation of the 22 restricted aromatic amines along with observed retention time, precision and linearity of the method is displayed in Table 2. The total ion chromatogram of positive control samples showing expected degradants are shown in figure 3.

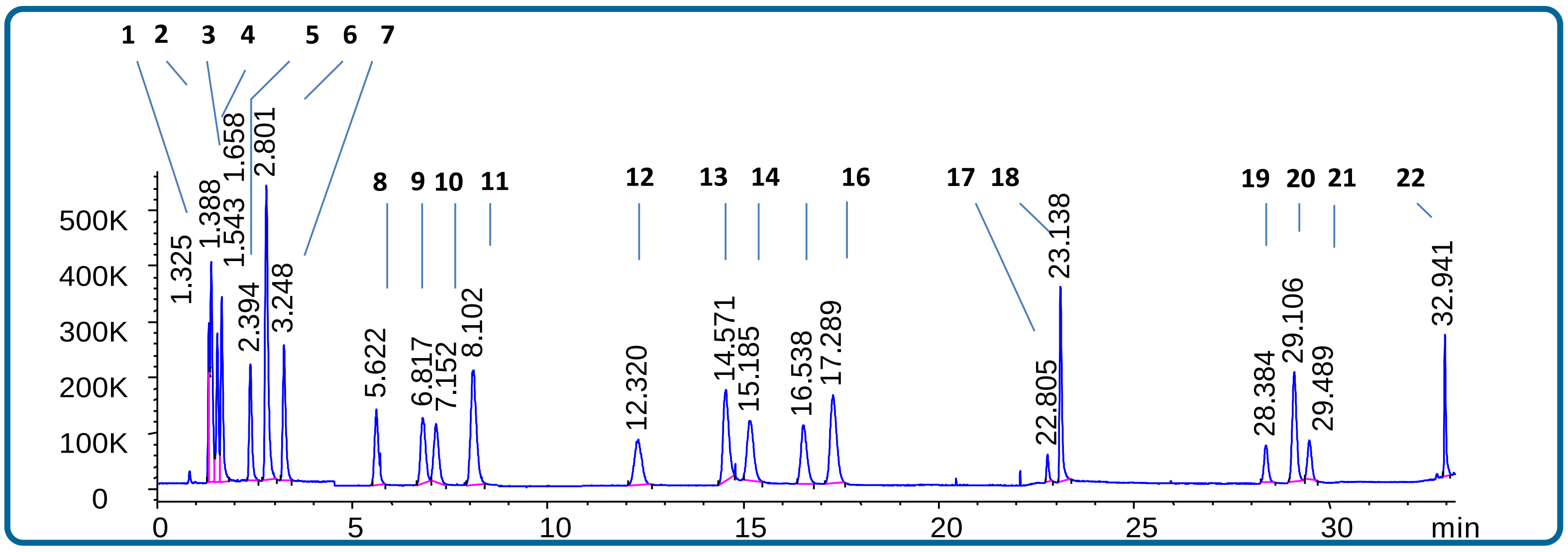

Figure 2: The LCMS method showing total ion chromatogram of mixture of 22 restricted aromatic amines

Compound Name	Abbreviated name	MW (M+H)$^+$	FV (V)	RT (min)	RSD of RT, (N =6)	RSD area, (N=6)	R^2 values
4-Methoxy-*m*-phenylenediamine	1	139.1	82	1.3	0.06	2.3	0.984
2,4-Diaminotoluene	2	123.1	60	1.4	0.13	3.2	0.987
Benzidine	3	185.1	98	1.5	0.1	1.5	0.991
4,4′-Oxydianiline	4	201.1	134	1.7	0.08	2.1	0.993
4,4′-Diaminodiphenylmethane	5	199.1	108	2.4	0.15	1.9	0.998
o-Anisidine (2-Methoxyaniline)	6	124.1	78	2.8	0.03	2.7	0.996
o-Toluidine	7	108.1	100	3.2	0.07	5.6	0.994
4-Chloroaniline	8	128.1	110	5.6	0.03	2.6	0.995
o-Tolidine	9	213.1	112	6.8	0.06	3.3	0.998
o-Dianisidine (3,3´-Dimethoxybenzidine)	10	245.1	90	7.2	0.04	1.8	0.996
2-Methoxy-5-methylaniline	11	138.1	90	8.1	0.08	2.6	0.998
4,4′-Methylene-bis(2-methylaniline)	12	227.3	128	12.3	0.11	3.4	0.999
2-Naphthylamine	13	144.1	92	14.6	0.03	4.9	0.999
4-Chloro-2-methylaniline	14	142.1	102	15.2	0.06	5.0	0.992
4,4′-Diaminodiphenyl sulfide	15	217.1	130	16.5	0.03	2.9	0.997
2,4,5-Trimethylaniline solution	16	136.1	112	17.3	0.04	4.8	0.996
2-Methyl-5-nitroaniline	17	153.1	110	22.8	0.01	2.8	0.997
4-Aminobiphenyl	18	170.1	138	23.1	0.01	2.6	0.999
3,3′-Dichlorobenzidine solution	19	253	114	28.4	0.01	2.4	0.995
4-Aminoazobenzene	20	198.1	98	29.1	0.02	2.0	0.999
4,4′-Methylene-bis(2-chloroaniline)	21	267	144	29.5	0.02	2.6	0.995
Fast Garnet GBC base	22	226.1	94	32.9	0.02	4.2	0.993

Table 2: Name and abbreviation of the 22 restricted aromatic amines. Compounds 1-7, 8-11, 12-16, 17-22 are grouped into time segments to increase specificity of the method. Accuracy of the method is within 20%.

Positive control samples

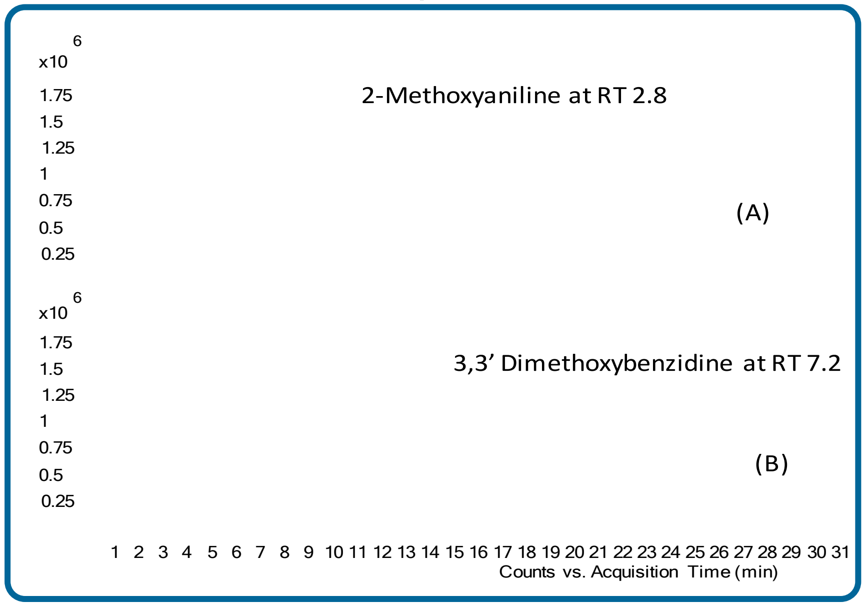

Figure 3: Textiles samples dyed with Acid Red 4 and Direct Blue 15 azo dyes followed by degradation and extraction procedures show the presence of compound 6 (figure 3A) and 10 (figure 3B) respectively as expected.

Results and Discussion

Recovery

Most amines showed a recovery value ~50% and is at par with literature results. Recovery results of 5 restricted aromatic amines from six polyester samples are as shown in Table 3.

Textile (spiking concentration)	#3	#4	#5	#9	#10
White polyester (0.39 pp)	39 ± 3	45 ± 3	33 ± 2	46 ± 2	44 ± 2
White polyester (0.79 ppm)	34 ± 5	40 ± 8	31 ± 5	40 ± 3	40 ± 5
White polyester (1.6 ppm)	27 ± 1	30 ± 2	25 ± 2	38 ± 2	36 ± 2
Textile (0.39 ppm)	40 ± 8	41 ± 15	31 ± 13	41 ± 15	39 ± 15
Textile (0.79 ppm)	40 ± 6	37 ± 14	29 ± 12	47 ± 13	40 ± 11
Textile (1.6 ppm)	37 ± 5	38 ± 12	31 ± 11	43 ± 8	40 ± 7

Table 3: Recovery of five restricted aromatic amines (3,4,5,9 and 10) from white polyester and colored textile samples at three different concentrations each. (n=3 for each samples).

Quantification of amines using standard addition method

Standard addition method induces a standard of the target compound directly into the analytical sample. In textile sample analysis, presence of wide variety of dyes and corresponding reduced products makes the sample very complex and standard addition method is suitable. The linearity curve constructed with use of standard addition method is demonstrated for one compound in figure 4.

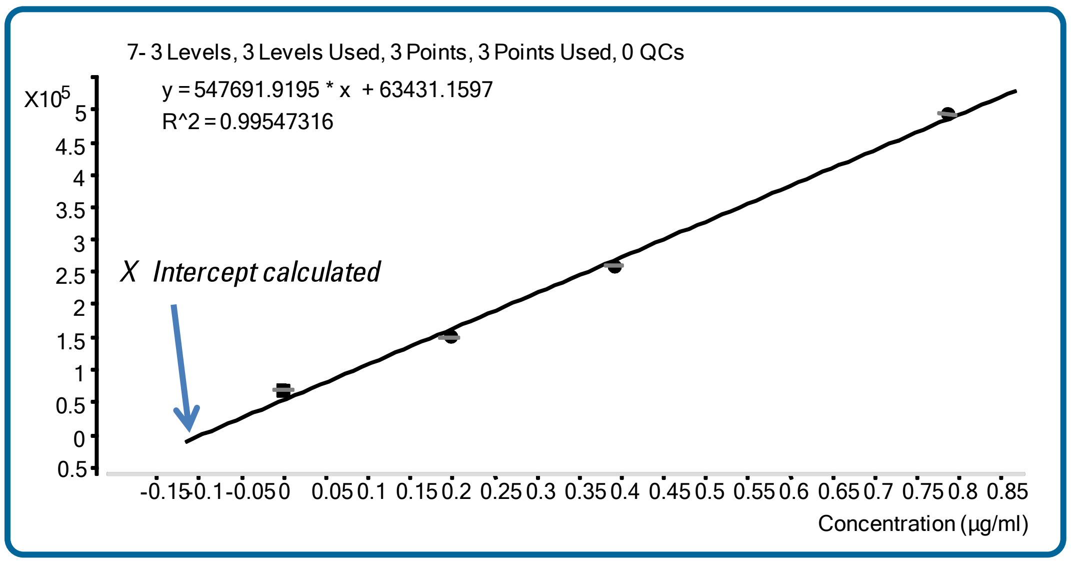

Figure 4: Linearity curve for compound #7 extracted from colored polyester textiles sample. X intercept (absolute) provides the concentration value in the unknown.

Results of analysis using standard addition method

Quantification of restricted amines in textile samples using standard addition method is compared against the external calibration standard method and tabulated in Table 4.

Textile samples	Concentration calculated using Standard addition method	Concentration calculated using external standard method
Textile 1 Compound 3	0.07 ppm	0.002 ppm
Textile 1 Compound 7	0.12 ppm	0.02 ppm
Textile 1 Compound 8	0.06 ppm	0.03 ppm
Textile 1 Compound 9	0.04 ppm	0.001 ppm
Textile 1 Compound 11	0.04 ppm	0.01 ppm
Textile 2 Compound 14	0.08 ppm	0.01 ppm

Table 4: The quantification results of some of six restricted amines using standard addition method and external standard method. The results from standard addition are closer to actual values.

Conclusions

- *LCMS method developed to separate 22 restricted aromatic amines. The method shows <0.1 RSD in retention time and <5.6 RSD is area response. Aqueous samples show excellent linearity over 6 concentration levels.*
- *Textiles samples dyed with Acid Red 4 and Direct Blue 15 showed as expected compounds 6 and 10 respectively.*
- *Extraction method using solid-liquid extraction was optimized to determine that ethyl acetate effectively extracts out all the compounds*
- *Although the reductive method using sodium dithionite in NaOH is used in literature, results show that recovery is very low in this reductive method. Improvements are required to optimize this method.*
- *Standard addition method provides effective method to quantitate textiles samples. Since the spiking standard is to be more than analyte present, some preliminary experiments may be required to determine if dilution is required.*
- *MassHunter quantitative software effectively calculates the unknown concentration using standard addition method.*

References

1. *Directive 2007/51/EC of the European Parliament and of the Council of 25 September 2007.*
2. *L.H. Ahlstrcom, C.S. Eskilsson, E. Bjcorlund, " Determination of banned azo dyes in consumer goods ," Trends Anal Chem, 24: 49-56, 2005*