

Analysis of Aromatic Amines Derived From Banned Azo Dyes in Textiles by CE-MS/MS

Application Note

Authors

Claudimir Lucio do Lago
Department of Fundamental Chemistry,
Institute of Chemistry,
University of São Paulo, Brazil

Vagner Bezerra dos Santos
Institute of Exact and Natural
Sciences,
Federal University of Pará, Brazil

Júlia Baruque-Ramos and
Wânia Duleba
School of Arts, Sciences and
Humanities,
University of São Paulo, Brazil

Welton Fernando Zonatti
Federal Institute of Education, Science
and Technology of Rio de Janeiro,
Brazil

Daniela Daniel
Agilent Technologies, Inc.
Materials Testing and Research

Abstract

In the textile industry, detecting aromatic amines derived from banned azo dyes is important in chemical safety control. To that end, we have developed a method using capillary electrophoresis in tandem with mass spectrometry (CE-MS/MS) for the determination of 4,4'-oxydianiline, 4,4'-diaminodiphenylmethane, and 4-chloro-2-methylaniline in fabric samples. The fabric samples were submitted to a modified QuEChERS extraction procedure, followed by electrophoretic separation in 0.5 M acetic acid electrolyte (pH 2.5) using a polyvinyl alcohol (PVA)-coated capillary. The determination coefficients of the calibration curves in the range of 10 to 1,000 ppb were up to 0.999, with limits of detection lower than 0.9 ppb for all analytes. We verified precision and accuracy through recovery for spiked samples at three concentration levels (10, 20, and 50 ppb), in triplicate measurements. The recovery values ranged from 92 to 123 %, with a relative standard deviation lower than 6.1 %.



Agilent Technologies

Introduction

Azo dyes are synthetic organic colorants that have been extensively used in numerous industrial applications, mainly because of their colorfastness and low price. However, whereas azo dyes are relatively resistant to degradation under aerobic conditions, they can be readily reduced to form aromatic amines under anaerobic conditions. A number of the aromatic amines formed have been found to be carcinogenic [1,2]. For this reason, the European Union has enacted legislation to prevent exposure to these hazardous amines, prohibiting the manufacture and sale of consumer goods containing certain aromatic amines originating from specific azo dyes [3]. According to this legislation, the concentration of any of these amines should not exceed 30 ppm in the finished articles or in the dyed parts thereof. Countries such as the United States have more restrictive laws about the content of these aromatic amines [4].

As a result, the textile industry needs efficient methodologies to quantify aromatic amines derived from banned azo dyes to ensure safety. Different analytical methods for detecting aromatic amines in fabric samples have been reported in various studies [5,6]. In this application note, we developed a method that uses capillary electrophoresis in tandem with mass spectrometry. This CE-MS/MS method was evaluated for the determination of three aromatic amines: 4,4'-oxydianiline, 4,4'-diaminodiphenylmethane, and 4-chloro-2-methylaniline (Figure 1).

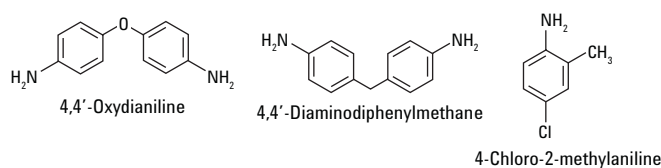


Figure 1. Molecular structure of aromatic amines.

Experimental

CE Conditions

Parameter	Value
Instrument	Agilent 7100 CE system
Background electrolyte	0.5 M acetic acid, pH 2.5
Applied voltage	28 kV
Capillary	PVA-coated silica capillary 50 μ m id with 60 cm total length (p/n G160U-61219, 125 cm length, cut to 60 cm)
Injection	15 seconds at 50 mbar
Temperature	25 °C

MS Conditions

Parameter	Value
Instrument	Agilent 6430 MS system
Ion mode	ESI, positive ionization
Sheath liquid	BGE solution diluted 10x with H ₂ O/methanol (50:50 v/v)
Flow rate	5.0 μ L/min
Capillary voltage	4,000 V
Drying gas flow (N ₂)	5 L/min
Drying gas temperature	250 °C
Nebulizer pressure	4 psi

All separations were performed at 25 °C using a 0.5 M acetic acid, pH 2.5, as background electrolyte (BGE). The sheath liquid used in the ESI was prepared by diluting the BGE 10x with H₂O/methanol 50:50 (v/v). New PVA-coated capillaries were preconditioned by flushing with Milli-Q water for 3 minutes followed by BGE for 5 minutes. We included an extra post-conditioning step by flushing with BGE for 60 seconds between the runs. Samples were introduced hydrodynamically for 15 seconds at 50 mbar, and analyzed at 28 kV. The mass spectrometer was operated in positive ionization mode, using multiple reaction monitoring (MRM) for two specific transitions for each aromatic amine. The most intense transition was used for quantification, and the other was used as a qualifying ion.

Table 1. Migration Time (t_M) and MS/MS Acquisition Parameters Used for the Identification and Quantification of Aromatic Amines in Fabrics

Analyte	t_M (min)	Q1 ^a (m/z)	Q3 ^b (m/z)	CE ^c (V)	FE ^d (V)
4,4'-Oxydianiline	3.94	201.1	184.1*	17	124
			93.0	41	
4,4'-Diaminodiphenylmethane	3.96	199.1	106.1*	25	144
			89.1	65	
4-Chloro-2-methylaniline	5.58	142.0	107.3*	16	120
			89.2	36	

^a Precursor ion (Q1); ^b fragment ions (Q3); ^c collision energy, and ^d fragmentor energy.

* Transition used for quantitation.

Sample preparation

We used four textile samples: red taffeta and blue taffeta (both 100 % polyester, canvas 1 × 1, 115 g/m², Asiatic origin), virgin indigo denim (381 g/m²), and denim (300 g/m²) of post-consumption trousers (both 100 % cotton twill 3 × 1, Brazilian origin). The samples were finely divided with the aid of scissors. Extraction of the aromatic amines from fabrics was performed using a modified QuEChERS method. This method involved placing a 1.0-g aliquot of the sample into a 50-mL PP tube followed by the addition of 10 mL of Milli-Q water, and extraction using 10.0 mL of acetonitrile (containing 10 mg of NaOH, apparent pH 12.4). A partition step was performed by adding 4 g of anhydrous magnesium sulfate (MgSO₄) and 1 g of anhydrous sodium chloride (NaCl) using Agilent original nonbuffered QuEChERS extraction tubes (p/n 5982-5550), followed by shaking for 1 minute, and centrifugation for 5 minutes at 5,000 rpm. Next, a 2-mL aliquot of the supernatant was filtered through a 0.2- μ m PVDF and PP membrane (Agilent Captiva filter cartridges, p/n A5300002), diluted with BGE, and analyzed. The dSPE cleanup step was unnecessary.

We carried out the recovery tests by spiking the samples with a known amount of the analytes, then allowing them to dry before the shaking step. This spiking resulted in three different levels of aromatic amines (0.01, 0.02, and 0.05 ppm) in the fabric samples. The recovery was calculated for each analyte as the average peak area of aromatic amine found in the spiked blank sample with the response of the same analytes from post-extracted samples at the equivalent concentrations, and was expressed as a percentage.

Results and Discussion

BGE and sheath liquid composition, applied potential, and hydrodynamic injection were optimized to achieve a good compromise between separation efficiency, sensitivity, and analysis time. A PVA-coated capillary (p/n G1600-67219) was used to achieve a good compromise between analysis time and peak resolution by reducing the electro-osmotic flow (EOF). The PVA coating minimized the interaction between highly polar compounds and the surface of the capillary to avoid excessive peak tailing. Figure 2 shows the normalized MRM electropherogram of aromatic amine standards in BGE using a PVA-coated capillary. The migration time for all aromatic amines was lower than 6.0 minutes.

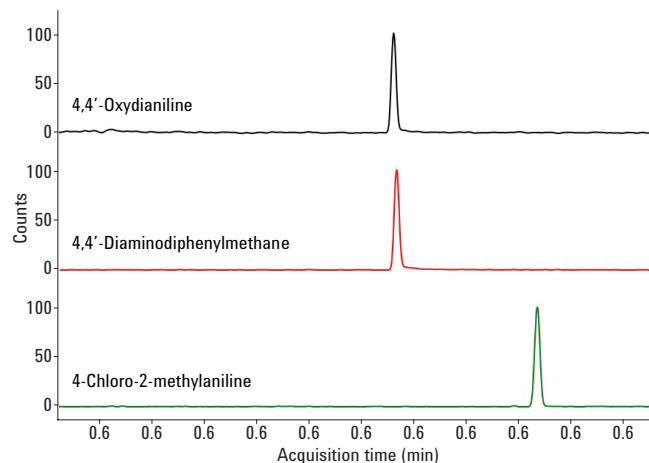


Figure 2. CE-MS/MS normalized electropherogram of a mixture of aromatic amines, 4,4'-oxydianiline, 4,4'-diaminodiphenylmethane, and 4-chloro-2-methylaniline, at 50 ppb each in BGE.

Figure 3 shows the linearity of the analytical curves studied in BGE at seven different concentration levels ranging from 10 to 1,000 ppb using MassHunter quantitative software. Calibration curves were constructed using matrix-matched aromatic amine standard solutions at five different concentration levels ranging from 10 to 100 ppb. The response function was found to be linear, with coefficient of determination (R^2) values higher than 0.997 for all calibration curves. The limits of detection (LOD) and limits of quantification (LOQ) were determined, considering the corresponding concentration, to produce a signal 3 and 10 times, respectively, the baseline noise in a close region to the migration time of each aromatic amine. The proposed method enabled us to detect aromatic amines at levels between 0.5 and 0.9 ppb. Table 2 shows some results for the developed method.

Table 2. Figures of Merit for the Determination of Aromatic Amines in Fabric Samples

Compound	$y = ax + b$	R^2	LOD (ppb)	LOQ (ppb)
4,4'-Oxydianiline	$y = 26594.7x + 69.6$	0.999	0.9	2.9
4,4'-Diaminodiphenylmethane	$y = 50903.1x - 81.6$	0.999	0.5	1.7
4-Chloro-2-methylaniline	$y = 29843.0x + 64.0$	0.999	0.6	2.0

Results obtained by analysis of variance (ANOVA).

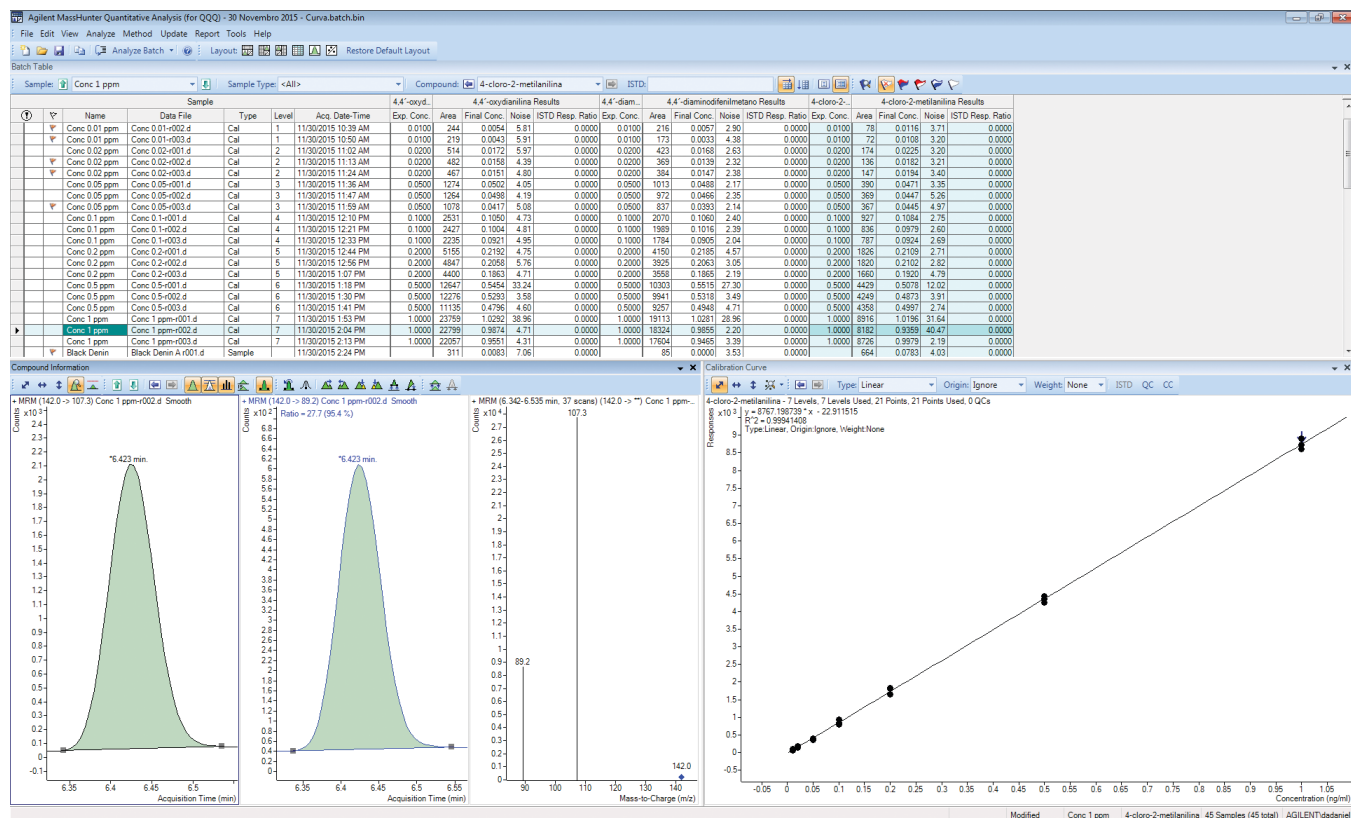


Figure 3. Agilent MassHunter quantitative software used for the determination of aromatic amines in fabric samples.

We analyzed each level of concentration in triplicate, and the run-to-run relative standard deviations (RSDs) ranged from 0.8 to 5.8 %. The standard addition calibration method was used in the determination of aromatic amines in fabric samples. Precision and accuracy, expressed in terms of recovery from fabric samples, were obtained by analyzing spiked samples at three different concentration levels (10, 20, and 50 ppb), in triplicate. The recovery values for samples spiked at three concentration levels ranged from 92 to 123 %, with standard deviation not greater than 6.1 % for triplicate analyses.

Table 3. Aromatic Amines in Fabric Samples by CE-MS/MS Expressed in $\mu\text{g}/\text{kg}$ of Fabric (ppb) and Recovery Tests Carried Out on These Samples ($n = 3$)

Analyte	Sample	Conc. added (ppb)	Conc. found (ppb)	Recovery (%)	Concentration (ppb)
4,4'-Oxydianiline	Red taffeta	10.0	9.3	93	12.8 ± 0.8
		20.0	20.4	102	
		50.0	58.6	117	
	Blue taffeta	10.0	12.3	123	ND
		20.0	21.9	109	
		50.0	50.6	112	
	Virgin denim	10.0	9.3	93	ND
		20.0	19.8	99	
		50.0	57.4	115	
	Used denim	10.0	10.4	104	ND
		20.0	19.3	96	
		50.0	48.3	97	
4,4'-Diaminodiphenylmethane	Red taffeta	10.0	9.8	98	10.6 ± 0.5
		20.0	21.9	110	
		50.0	60.9	122	
	Blue taffeta	10.0	10.4	104	<LOQ
		20.0	23.2	116	
		50.0	50.4	101	
	Virgin denim	10.0	9.9	99	ND
		20.0	21.4	107	
		50.0	57.0	114	
	Used denim	10.0	11.4	114	ND
		20.0	21.0	105	
		50.0	54.4	109	
4-Chloro-2-methylaniline	Red taffeta	10.0	10.3	103	ND
		20.0	21.7	109	
		50.0	55.2	110	
	Blue taffeta	10.0	11.6	116	25.2 ± 1.5
		20.0	20.6	103	
		50.0	46.0	92	
	Virgin denim	10.0	10.4	104	16.7 ± 0.7
		20.0	24.5	122	
		50.0	52.2	104	
	Used denim	10.0	10.9	109	ND
		20.0	24.4	122	
		50.0	49.6	99	

< LOQ, lower than quantitation limit; ND, not detected.

Conclusion

This application note shows that CE-MS/MS is suited for the analysis of aromatic amines in textile samples. The use of a PVA-coated silica capillary provided EOF suppression and increased separation efficiency with no peak tailing effects. The proposed method presented a linear response to aromatic amines in the concentration range of 10 to 1,000 ppb, with an LOD lower than 0.9 ppb. The modified QuEChERS extraction (at high pH) was simple and efficient with good recovery values. In addition, the method is simple, fast (less than 6 minutes per sample), used a small amount of sample with low reagent consumption, and showed good sensitivity and precision. The highest residue found in the textile samples analyzed was 25.2 ppb, several orders of magnitude below the maximum allowed value in the European Parliament Directive 2002/61/EC and FDA recommendations [3,4]. These results indicate that CE-MS/MS is a perfectly eligible technique for aromatic amines analysis in textile samples.

References

1. L.-H. Ahlström, C. S. Eskilsson, E. Björklund. "Determination of banned azo dyes in consumer goods." *Trends in Analytical Chemistry* **24**, 49-56 (2005).
2. H. M. Pinheiro, E. Touraud, O. Thomas. "Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters" *Dyes and Pigments* **61**, 121–139 (2004).
3. Directive 2002/61/EC of the European Parliament and of the Council (19 July 2002) amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azo colorants).
4. Guidance for Industry: Color Additive Petitions - FDA Recommendations for Submission of Chemical and Technological Data on Color Additives for Food, Drugs, Cosmetics, or Medical Devices.
5. M. Huang, R. Fu, Analysis of EU banned azo colorants in textiles using Poroshell 120 and 1290 Infinity, *Agilent Technologies Application Note*, publication number 5990-7705EN (2011).
6. R. G. Dall'Anese, G. Bartolini, A. Franchi, 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, *Agilent Technologies Application Note*, publication number 5991-5169EN (2014).

Acknowledgements

The authors gratefully acknowledge financial support from Brazilian foundations FAPESP (2012/06642-1).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 2017
Printed in the USA
February 10, 2017
5991-7831EN



Agilent Technologies