# Analysis of EU banned Azo colorants in textiles using Poroshell 120 and 1290 Infinity

Application Note

Environmental


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## Abstract

22 restricted aromatic amines and four azo compounds derived from azo colorants were analyzed using a Agilent Poroshell 120 EC-C18 and Agilent 1290 Infinity LC System. The new method resolved the 26 aniline compounds in only 14 min with ruggedness and reproducibility.


## Introduction

Azo dyes are a large class of synthetic organic dyes that contain nitrogen as the azo group -N=N- as part of their molecular structures. They are often used in the coloring process of textiles and leather. Some of these dyes have the capacity to release certain aromatic amines, which pose cancer risks. For this reason, the EU has released legislation to prevent exposure to these hazardous amines. Regulation EN14362 [1, 2] prohibits the use of azo dyes that may release one of the 22 aromatic amines as listed in the Table 1 (not including compounds No. 1,4,13 and 14). This prohibition applies to all textile and leather products that may come into direct and prolonged contact with the human skin or mouth.

In this note, 22 EU banned aromatic amines with an additional 4 compounds (1, 4-phenylendiamine, aniline, 2,6-dimethylaniline and 2,4-dimethylaniline) were analyzed using an Agilent Poroshell 120 EC-C18, 3.0 × 150mm, 2.7 µm column and an Agilent Infinity 1290. The gradient was modified from the method in EN 14362 to enhance resolution with this column.

*Table 1. List of Azo-dyes for Analysis (Compounds No. 1, 4, 13 and 14 are Not Included in EU Method)*

| No. | Name | CAS No. |
|---|---|---|
| 1 | 1,4-phenylendiamine | 106-50-3 |
| 2 | 4-methoxy-m-phenylenediamine | 615-05-4 |
| 3 | 4-methyl-m-phenylenediamine | 95-80-7 |
| 4 | Aniline | 62-53-3 |
| 5 | benzidine | 92-87-5 |
| 6 | o-anisidine<br>2-methoxyaniline | 90-04-0 |
| 7 | 4,4'-oxydianiline | 101-80-4 |
| 8 | o-toluidine<br>2-aminotoluene | 95-53-4 |
| 9 | 4-chloroaniline | 106-47-8 |
| 10 | 5-nitro-o-toluidine | 99-55-8 |
| 11 | 4,4'-methylenedianiline<br>4,4'-diaminodiphenylmethane | 101-77-9 |
| 12 | 6-methoxy-m-toluidine<br>p-cresidine | 120-71-8 |
| 13 | 2,6-dimethylaniline | 87-62-7 |
| 14 | 2,4-dimethylaniline | 95-68-1 |
| 15 | 3,3'-dimethoxybenzidine<br>o-dianisidine | 119-90-4 |
| 16 | 3,3'-dimethylbenzidine<br>4,4'-bi-o-toluidine | 119-93-7 |
| 17 | 4,4'-thiodianiline | 139-65-1 |
| 18 | 2-naphthylamine | 91-59-8 |
| 19 | 4-chloro-o-toluidine | 95-69-2 |
| 20 | 2,4,5-trimethylaniline | 137-17-7 |
| 21 | 4,4'-methylenedi-o-toluidine | 838-88-0 |
| 22 | biphenyl-4-ylamine<br>4-aminobiphenylxenylamine | 92-67-1 |
| 23 | 3,3'-dichlorobenzidine<br>3,3'-dichlorobiphenyl-4,4'-ylenediamine | 91-94-1 |
| 24 | 4-aminoazobenzene | 60-09-3 |
| 25 | 4,4'-methylene-bis-(2-chloro-aniline)<br>2,2'-dichloro-4.4'-methylene-dianiline | 101-14-4 |
| 26 | o-aminoazotoluene<br>4-amino-2',3-dimethylazobenzene<br>4-o-tolylazo-o-toluidine | 97-56-3 |

# Experimental

## Instrument and Reagents

The Agilent Infinity 1290 LC System includes a binary pump, a thermostatted column compartment (TCC), a high performance autosampler and a diode array detector (DAD).

The column used in the application is an Agilent Poroshell 120 EC-C18 3.0 × 150 mm, 2.7 µm (p/n: 693975-302).

The 26 standards (Table 1) were purchased from J&K (Beijing, China). The mixture was dissolved in methanol and mobile phase A (10:90) (see conditions) to a concentration of 12 µg/mL and stored at 4 °C in a dark area.

## HPLC conditions

| | |
|---|---|
| Column | Poroshell 120 EC-C18, 3.0 mm × 150 mm, 2.7 µm (p/n: 693975-302) |
| Column Temp | 40 °C |
| Flow Rate | 0.8 mL /min |
| Mobile Phase | A: 0.575 g Mono ammonium phosphate, 0.7 g Disodium hydrogen phosphate in 900 mL water added with 100 mL methanol and adjusted using phosphoric acid to pH 6.9<br>B: methanol |
| DAD Wavelength | 240 nm; 280 nm; 305 nm; |
| Injection volume | 1 µL |

# Results and discussion

## Separation options

The EU method uses a 4.6 × 250 mm, 5 µm column for the analysis and a long gradient of 70 min. [1] Two phases of Poroshell 120 including SB-C18 and EC-C18 were tested for the method. The Poroshell 120 EC-C18 provides excellent selectivity and peak shape for these aniline compounds. The 2.7 µm superficially porous particles provide similar performance to sub-2 µm totally porous particles, and a 150 mm length column gives a very high peak capacity. This complex mixture of samples needs high peak capacity for better resolution in less analysis time. Of this mixture, 4, 4'-methylenedianiline (11), 6-methoxy-m-toluidine (12), 2,6-dimethylaniline (13), 2,4-dimethylaniline (14), 3,3'-dimethoxybenzidine (15) and 3,3'-dimethylbenzidine (16) are difficult to separate. They are sensitive to slight changes in the gradient. The gradient in Table 2 was modified to get an optimized separation. The chromatogram shown in Figure 1 was achieved using this modified gradient. All 26 compounds are separated well in 15 min, which is nearly 80% faster than the recommended method.

A smaller internal diameter of 3.0 mm saves around 60% of solvent compared to that of the 4.6 mm id column.

Many of these azo dyes have their own maximum UV absorption; therefore, multiple wavelengths including 240 nm, 280 nm and 305 nm were used for detection to achieve good signals for each peak according to the EU method.

*Table 2. Modified Gradient for 26 Standards Separation*

| Time (min) | B% |
|---|---|
| 0 | 14 |
| 4.5 | 29 |
| 8 | 29 |
| 9 | 50 |
| 10.5 | 65 |
| 12 | 90 |
| 14 | 100 |
| 15 | 14 |

## Reproducibility and ruggedness

The 2.7 µm superficially porous particles have similar performance to 1.8 µm particles, but with 40% lower back pressure than the 1.8 µm columns. Even on a length of 150 mm column, the maximum back pressure is 580 bar and the method can still be run on a 600 bar limit UHPLC. In this note, a longer Poroshell120 EC-C18 (3.0 mm × 150 mm, 2.7 µm) column coupled with Agilent Infinity 1290 gives us more flexibility to achieve even greater resolution quickly and very reliable results. An overlay chromatogram in Figure 2 shows excellent reproducibility of the method. The overlays include chromatograms generated on two different days with two different batches of mobile phase.

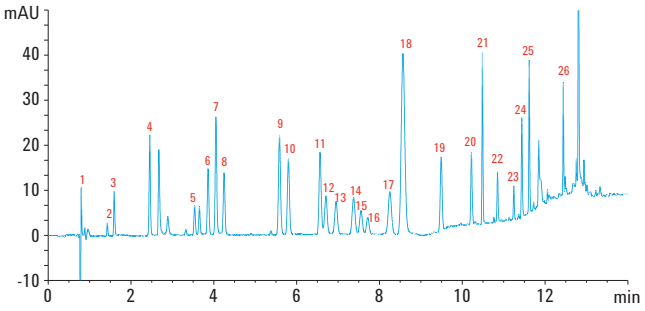


*Figure 1. Chromatogram of 26 azo-dyes standards on Agilent Poroshell 120 EC-C18, 3.0 mm × 150 mm, 2.7 µm at 240 nm.*

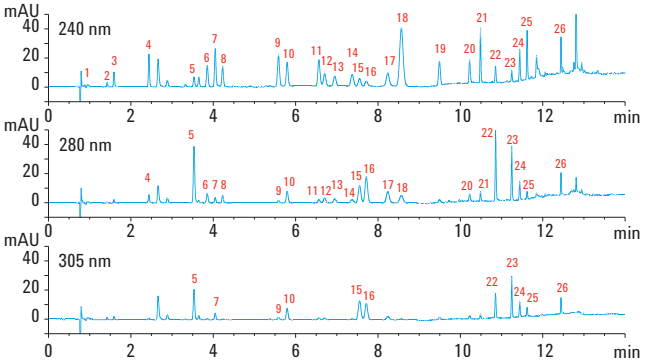


*Figure 2. Chromatograms at UV 240 nm, 280 nm and 305 nm.*

## Results

The method developed on a new superficially porous particle column, the Agilent Poroshell 120 EC-C18, 3.0mm × 150mm, 2.7 um, resolved 26 azo-dyes with baseline separations within 15 minutes. The method coupled with the new Agilent Infinity 1290 gives excellent reproducibility and reliable results. Significant time (nearly 80%) and solvent savings (nearly 60%) were achieved for this method.

## Reference


1. EN 14362-1-2003 Textiles - Methods for determination of certain aromatic amines derived from azo colorants. Part 1: Detection of the use of certain azo colorants accessible without extraction.
2. EN 14362-2-2003 Textiles. Methods for the determination of certain aromatic amines derived from azo colorants. Part 2: Detection of the use of certain azo colorants accessible by extracting the fibres.


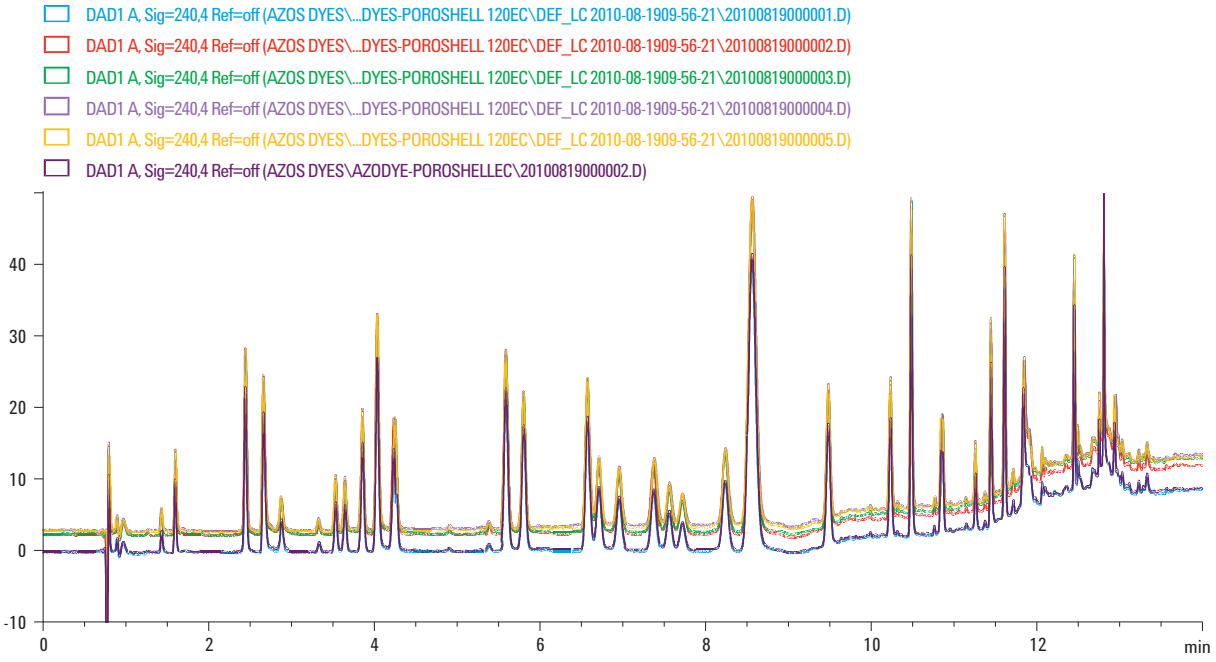


*Figure 3. Overlay chromatograms of six injections at 240 nm on two different days with two different batches of mobile phase.*

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