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## Detection of azo dyes and aromatic amines in women under garment

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### Abstract

Women are exposed to several chemical additives including azo dyes that exist in textile materials that are a potential health hazard for consumers. Our objective was to analyze suspected carcinogenic azo dyes and their degradation aromatic amines in women's panties underwear using a fast and simple method for quantification. Here, we evaluated 120 different samples of women underwear for their potential release of aromatic amines to the skin. Seventy four samples yielded low level mixtures of aromatic amines; however eighteen samples were found to produce greater than 200 mg/kg (ppm) of aromatic amines. Azo dyes in these 18 samples were extracted from the fabrics and analyzed by reverse phase thin layer chromatography in tandem with atmospheric pressure chemical ionization mass spectrometry.

Eleven azo dyes were identified based on their mass spectral data and the chemical structure of the aromatic amine produced from these samples. We demonstrate that planar chromatography and mass spectrometry can be really helpful in confirming the identity of the azo dyes, offering highly relevant molecular information of the responsible compounds in the fabrics. With the growing concern about the consumer goods, analysis of aromatic amines in garments has become a highly important issue.

### Keywords

Fabrics; carcinogens; women underwear; aromatic amines; dermal toxicity; HPTLC/MS

## INTRODUCTION

Worldwide consumption of dyes is around  $7 \times 10^4$  tons per year, about 70% of which are azo-dyes, constituting the largest group of dyes used in industry.<sup>[1]</sup> This class of dyes is characterized by an azo bond ( $R-N=N-R$ ) that allows visible light to be absorbed by the dyes as a visible light chromophore. These dyes offer a broad spectrum of colors and are therefore used for coloring a large variety of consumer goods, such as leather, clothing, food, toys, medical devices, plastics, tattoo inks and cosmetics.<sup>[2]</sup> At present, three thousand azo dyes are used worldwide and they constitute more than 65% of the global commercial dye market.<sup>[3]</sup> When azo dyes are used in coloring underwear they are directly contact human

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skin for several hours on a daily basis <sup>[4]</sup>, causing possible dermal absorption through sweat and under certain conditions some azo dyes can break up and release aromatic amines (e.g., when they are reduced by azoreductases in intestinal bacteria microflora, liver cells, extra hepatic tissue, and epidermal cells <sup>[5, 6]</sup>, some of which are potential or proven carcinogenic agents. <sup>[7–11]</sup> Several epidemiological studies have demonstrated that the exposure to azo-based dyes has caused cancer in humans. <sup>[12–14]</sup> Although the direct determination of azo dyes has been explored <sup>[15–17]</sup>, because of the large number of possible dyes and the combined use of several dyes in industrial applications, determination of the aromatic amines produced after the reduction of the dyes is a more efficient method. <sup>[18–20]</sup> Irrespective of the dye or the combination of dyes, the presence of targeted aromatic amines in the reduced extract indicates the presence of one or more banned azo dyes in the sample. European Union (EU) in 2002 had restrictions on marketing and the use of azo dye and established a limit of less than 30 µg/g. <sup>[21]</sup> In China, 2, 4- and 2,6-xyldine were prohibited in China by the National Standard for the People's Republic of China (GB/T 17592-2006). <sup>[22]</sup> Here, we evaluated 120 different samples of women underwear for their potential release of aromatic amines to the skin either by microbial metabolism or body sweat formation, wear time and body activity.

## MATERIALS AND METHODS

### Chemicals and Reagents

All solvents used in this investigation were of HPLC/MS grade and purchased from J.T.Baker (USA), ammonium hydroxide was purchased from EM Science (USA). Diatomaceous Earth powder, and the 250 mL clear collection bottles were purchased from Thermo Fisher Scientific (USA), while extraction cells and glass fiber filters were purchased from Dionex (USA). Water HPLC grade was purchased from Fisher (USA), Sodium dithionite was purchased from Sigma (USA), and methyl alcohol anhydrous was purchased from Macron (USA). Potassium hydroxide was purchased from MCB (USA), citric acid monohydrate was purchased from Amresco (USA), and 1.8 mL auto Sampler vial was purchased from VWR (USA), filter papers was purchased from Baxter (USA), Nitrogen gas tank was obtained from Air Liquide (USA). C<sub>18</sub> silica HPTLC plates, w/UV 100% silanization, glass backed 200 nm, 10×10 cm was purchased from Sorbent Technologies (USA). 24 standard aromatic amines and 20 standard azo dyes were purchased from Sigma Aldrich (USA).

### Fabric Samples

120 collections of women underwear were purchased from department stores representing varieties in colors, fabrics structure (natural and synthetics), origin, manufactured geographical locations and brand names. Figure 1 shows number of samples collected according to their brand names, manufacturing countries, colors, fabric types and store names where samples were collected.

### Samples Extraction and Processing

Three replicates of each textile of 3 grams each was cut into small pieces followed by extraction with freshly prepared anhydrous methyl alcohol/potassium hydroxide (56.1mg/mL) 9:1 (v/v) using Dionex ASE 300 Accelerated Solvent Extractor. The extraction processes was carried out by inserting a glass fiber filter inside of a 34 mL-extraction cell, then 1g of diatomaceous earth powder (DE) was added followed by inserted 3g of textile sample, topped with more DE powder, and tightly packed it by using the insertion tool, then placed one glass fiber filter and covered it with the extraction cell lid. Extraction was then processed by performing a 5 minutes static heating, 5 minutes flush with 30% solvent, purge for 100 seconds for one cycle at a pressure of 1500 psi and a temperature of 100 °C. Total extraction time was 20 minutes per sample. Extracts were then cooled down to room temperature and volume was adjusted to 80 mL with extracting solvent. The extract volume was divided in to 2 portions, one 10 mL portioned was saved for direct spectroscopic and chromatographic analysis and the other portions of the 70 mL was used for the reduction process for obtaining aromatic amines.

### Determination of Aromatic Amines Produced by Azo Dyes

The general analytical procedure involved in the determination of aromatic amines produced by azo dyes involves three steps: reduction of extracted dyes using sodium dithionite in controlled conditions of acidity and temperature; extraction, cleanup, and pre-concentration of the amines, and the chromatographic separation and determination of analytes by gas chromatography mass spectrometry (GCMS).<sup>[23–25]</sup> Accelerated solvent extracts of each individual sample as described above was taken to dryness using rotatory evaporator, then transferred to reaction tube in 17 mL of pH 6 citrate buffer solution preheated to 70°C. Samples were thoroughly mixed using ultrasound bath and kept for 30 min at 70°C using heating blocks. 3 mL of aqueous sodium dithionite solution was then added and heated for additional 30 minutes with occasional shaking to cleave the azo dyes to their corresponding aromatic amines. The reaction tube was cooled to room temperature and extracted with 2×20 mL of methyl tertiary butyl ether (MTBE). The 2 combined MTBE layers were filtered over anhydrous sodium sulfate and concentrated to approximately 1 mL with a rotary evaporator keeping the temperature of the water bath below 30°C. Next, the volume of the sample solution was adjusted to 5 mL with MTBE and then analyzed by GC/MS.

### Gas Chromatography Mass Spectrometry (GCMS) Analysis

Samples extract that was reduced by sodium dithionite to release the aromatic amines as described above were analyzed using Agilent 7890B/5977A GCMSD system equipped with 7693A Automatic Liquid Sampler, Agilent fused silica 30 m × 0.25 mm, 0.25 µm capillary column. Helium was used as the carrier gas with gas flow rate of 1.0 mL/min. The temperatures of the injector, transfer line and ion source were 230°C, 250°C, and 230°C, respectively. 1 µL of the sample was injected in splitless mode, GC oven temperature was initially maintained at 55°C for 5 min and programmed to 250°C at a rate of 15°C/min. Mass spectra were acquired in the electron ionization mode at 70 eV. Twenty four aromatic amines standards were used to establish qualitative, and quantitative protocols. External standard and spiking samples with standard amines were used to calculate concentration of

aromatic amines in the sample. Chemical structures and total ion chromatogram of the 24 standard aromatic amines are shown in Figures 2 and 3 respectively. Quantitative analysis protocol was set up using serial dilution of 5 concentrations of the 24 standard aromatic amines. Total ion chromatogram (TIC) and relative response as a function of the five serial concentration of the standard analytical aromatic amines are shown in Figures 3 and 4 respectively. Mass Hunter software was used for establishing the limits of detection and quantification (LOD and LOQ) and was found to be 1.2 and 12.0 ppm ( $\mu\text{g/g}$  fabrics) respectively. Reaction recovery and reduction efficiency were calculated using 3 replicates of the azo dye Ponceau Xylidine (Acid Red 26, Chemical Abstracts Identification Number: 3761-53-3). The results show that reaction efficiency and recovery was greater than 85%. Detector respond and dynamic range for representative samples of aromatic amines are shown in Figure 4.

### High Performance Thin Layer Chromatography (HPTLC)

**HPTLC plates and mobile phases**—Square Nano-Sil  $\text{C}_{18}$  reverse phase HPTLC plates (10 cm $\times$ 10 cm, with UV indicator) were used in this investigation. The mobile phase consisted of acetonitrile, ammonium hydroxide 95:5 (v/v) for developing solvent.

### Sample application and chromatography

10  $\mu\text{L}$  samples were sprayed on the plates using a CAMAG Automatic TLC Sampler 4 (CAMAG, Muttentz, Switzerland) in bands (8 mm) 15 mm from the bottom edge of the plate. Chromatographic development was performed with a saturated automatic ADC2 Chamber (Twin Trough Chamber, CAMAG, Muttentz, Switzerland) to a distance of 85 mm from the bottom edge of the plate. Air humidity was adjusted by means of a saturated magnesium chloride (Merck, Darmstadt, Germany) solution. Plate preconditioning time for development was 5 minutes, and drying time was 5 minutes.

**Documentation**—Images at 254 nm, 366 nm and white light were recorded on a Reporter 3 with digital camera Baumer Optronic DXA252 (CAMAG, Muttentz, Switzerland). All data were analyzed with winCATS 1.4.6 (CAMAG, Muttentz, Switzerland).

**HPTLC/MS Analysis**—High performance thin layer chromatography/mass spectrometry was achieved by direct coupling using a TLC–MS interface equipped with the round elution head (CAMAG, Muttentz, Switzerland) enabling desorption of the analytes from the plate by an HPLC pump (Agilent 1100 Quaternary Pump, Agilent Technologies, USA) driven solvent stream. Analytes were extracted from the plate with acetonitrile 0.1% formic acid. Mass spectra of zone extracts was performed with an Agilent 6130 single quadrupole mass spectrometer operating in positive and negative atmospheric pressure chemical ionization (APCI) operated by Agilent OpenLab Control Panel A.01.04. Full scan MS data acquisition was carried out in both positive and negative mode with following settings: capillary voltage 3.0 kV, skimmer voltage 35 V, lens 2.5 V, quadrupole temperature 150°C, drying gas temperature 350°C, drying gas flow rate 10 L/min and nebulizer gas pressure 60 psig. Spectra were recorded in the ranges of 200–900  $m/z$ , with fragmentor voltage 100 V, gain 1, threshold 100, and step size 0.25, corona (positive) at 6.0  $\mu\text{A}$  and corona (negative) at 6  $\mu\text{A}$ .

Structural identification of the azo dyes in each of the TLC band for each sample was based on using the designated resulted aromatic amine as the specific moiety for the azo dye and mass spectral data in the form of molecular ions ( $M^+$ ,  $M+H^+$  or  $M+Na^+$ ) for positive ions or ( $M^-$ ,  $M-H^-$ ) for negative ions. The results were identified using azo dyes database for the corresponding molecular ions SciFinder® structural search for the azo dyes group.

## RESULTS AND DISCUSSION

### GCMS Quantitative Analysis of Aromatic Amines

Quantitative analysis of aromatic amines of the 120 samples was carried out using accelerated solvent extraction followed by reduction with sodium dithionite as described in the experimental section. No aromatic amines were detected in 46 of the 120 samples, indicating the absence of any of the azo dyes. 74 samples showed the presence of one or more aromatic amines at concentration levels greater than 2  $\mu\text{g/g}$  (ppm), 18 samples out of the 74 samples showed a level of aromatic amines exceed the regulation limits recommended by international organizations of 20 ppm. Total ion chromatograms of all of the 18 samples are presented in Figure 5 along their color, brand names, sources, country of manufacturing as well as the identified aromatic amines and their concentration expressed as the manufacturing as well as the identified aromatic amines and their concentration expressed as the average of three replicates and their standard deviation. As seen from Figure 5, all of the 18 samples produced only one aromatic amine except the brown sample produced 2 different but related aromatic amines. Out of the eighteen samples only one was blue color, 4 were black, one was brown, 6 were purple, 2 were pink and four were red.

With regards to type of fabrics, 6 were cotton, 10 were nylon and 2 were polyesters. It is interesting to find out that none of these samples were made in the USA and the majority were made in China and El Salvador. The highest concentration was in cotton fabrics ranging from 98 to 897  $\mu\text{g/g}$  with an average of 346  $\mu\text{g/g}$  followed by polyester ranging from 152 to 183  $\mu\text{g/g}$  with an average of 167  $\mu\text{g/g}$  and nylon fabrics were the lowest in their contents of aromatic amines with a range from 21 to 160  $\mu\text{g/g}$  averaging 71  $\mu\text{g/g}$ . Black fabrics were the highest in their concentration of aromatic amines followed by brown, red, purple, pink and blue colors with an average concentrations of 388, 152, 119, 98, 69 and 22  $\mu\text{g/g}$  respectively. Samples from Egypt showed the highest concentration of aromatic amines followed by samples made in Vietnam, Bangladesh, China, EL Salvador, India and Honduras with an average concentrations of 479, 298, 134, 113, 110, 57 and 22  $\mu\text{g/g}$  respectively. Summary of aromatic amines concentrations according to samples color, country of manufacturing and fabrics type is shown in Figure 6. Ten different primary aromatic amines were identified based on their GC retention time and mass spectral search using NIST 2011 and Wiley 2011 database.

### Reversed Phase RP-HPTLC Chromatography

The 18 fabric samples were subjected to reverse phase HPTLC separation provided complementary information about the dyes composition as shown in Figure 7. Sample #1 was made of nylon fabrics and was blue in color; it showed one weak blue band and strong

purple band. Samples 2, 3, 4 and 5 were cotton fabrics and all are black in color their TLC migration were similar, however, they showed two bands of orange and black. Sample # 6 which is the only brown polyester fabric showed 4 bands, but the major colors were red and blue which together appear as brown. Six samples were purple fabrics, four of them were nylon (samples # 8,9,10 and 11), one was cotton (sample # 7) and one was polyester (sample #12). Purple nylon fabrics showed unique TLC pattern for each sample with no apparent similarity. Purple cotton fabrics showed one major purple band, however, polyester purple fabrics showed three bands. The two pink samples (13 and 14) were made of nylon and showed similar mobility. In the four red samples, one was made of cotton (15) and the others were nylon (16, 17 and 18), all of the four samples showed different profiles profiling in the HPTLC.

### HPTLC-MS

The eighteen selected fabric samples were shown to produce 10 aromatic amines upon reduction with sodium dithionite indicating that the same aromatic amine may be produced from more than one sample. Chemical structure for the identified aromatic amines is shown in Figure 5. Chemical identification of the bands in the HPTLC chromatogram of fabrics extract was confirmed by mass spectrometry, i.e., by use of CAMAG® HPTLC-MS interface, which enables direct coupling of HPTLC to MS without scratching the spots from the sorbent. Figure 8 shows the mass spectrum of one of the HPTLC band of sample #1 as an example of the identification of the possible azo dye in the HPTLC band. The aromatic amine 2-(2-methylphenoxy)aniline was associated with samples # 1, 8, 11 and 13, and based on molecular ions from the mass spectral data for each HPTLC band, two azo dyes (1 and 2) were identified as described in the methodology section and are shown in Figure 9. The aromatic amine 4-(butylsulfonyl) aniline was found to be associated with samples # 2, 3, 4, 5 which are all black cotton fabrics. Only one azo black dye (4) belonging to this group was identified with its structure shown in Figure 9. It is interesting to find out that the four samples are made in different countries (India, Vietnam and Egypt), but showed the same type of azo dyes. The aromatic amine 2-chlorobenzene-1, 4-diamine was found to be associated with samples # 6 and 12. One azo dye (3) was identified in this group as shown in Figure 9. Samples # 6 and 12 were made of polyester fabrics and showed very similar TLC pattern with both sharing the dye. The aromatic amine 4-chloro-2-(trifluoromethyl) aniline was associated with samples #9, 10 and 16. Three azo dyes (6, 7 and 8) were identified in this group as shown in Figure 9. The three samples were made of nylon fabrics and although that they were made in India and El Salvador they showed identical contents of the three azo dyes (6, 7 and 8) as shown in Figure 9. The aromatic amine 1, 3-benzothiazol-2-amine was associated with sample #17 with one azo dye identified in the group (5) as shown in Figure 9. The aromatic amine *N,N*-diethylbenzene-1,4-diamine was associated with sample #15 with one azo dye identified in the group (11) as shown in Figure 9. The aromatic amine 4-(4-chlorophenoxy) aniline is associated with samples # 14 and 18 with one dye identified (10) as shown in Figure 9. The two samples were made of nylon fabrics and both were made in Bangladesh and showed similar composition of the azo dye. The aromatic amine 4-methoxy-3-(trifluoromethyl) aniline is associated with samples #7 which is made of cotton fabrics with one TLC band corresponding to the dye (9).



## CONCLUSIONS

Women are exposed to several chemical additives existing in textile materials in underwear panties due to daily direct contact with the fabric materials. Many of these chemicals such as dyes, metals, fire retardants and synthetic industrial additives may represent a health hazard for consumers, so it is important to make sure that they are as low as possible and to eliminate chemicals that may be known as carcinogenic or procarcinogenic. Many of the azo dyes are belong to that group of health hazardous substances. A fast and simple method for quantification of the azo dyes in textile materials is described in this paper.

Twenty-four primary aromatic amines (PAAs) originated from azo dyes in commercial women underwear panties (120 samples) that are used in the United States were analyzed. 18 samples out of the 120 investigated samples (15%) were shown to produce higher level of aromatic amines more than what have been recommended by the European Union (EU) or China, USA has yet to establish recommended levels. The highest level of aromatic amines were mostly associated with cotton fabrics, black colors and made in Egypt items. The use of planar chromatography and mass spectrometry can be really helpful in confirming the identity of the azo dyes, offering highly relevant molecular information of the responsible compounds in the fabrics. In this work, the direct combination of HPTLC and APCI ionization mass spectrometry was facilitated by the TLC-MS interface. Target compounds were eluted from the HPTLC plates and directly introduced into the ion source. The developed method based on a simple and rapid extraction without any further clean-up of extracts. Compared to traditional LC-MS methods, the simplicity and efficiency of the method implied a great reduction of work with scaled-up screening tasks. Thus, it is an attractive alternative to LCMS for the rapid screening of azo dyes in fabrics. It is explicitly reported that the use of azo dyes is forbidden if those build banned amines through fragmentation of one or more azo groups. With the growing concern about the consumer goods, trace-level analysis of aromatic amines in garments has become highly important in recent years.

## Acknowledgments

### FUNDING

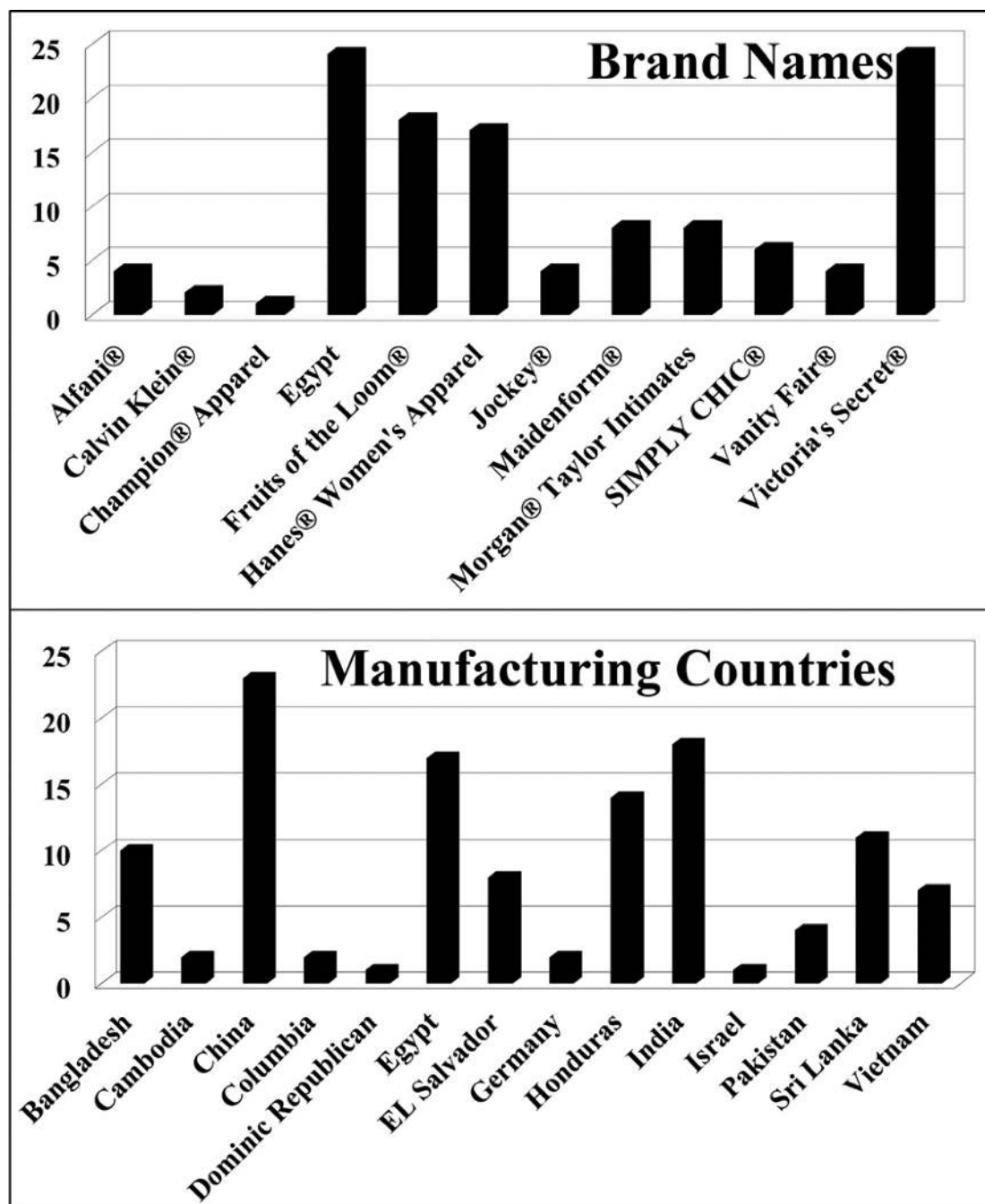
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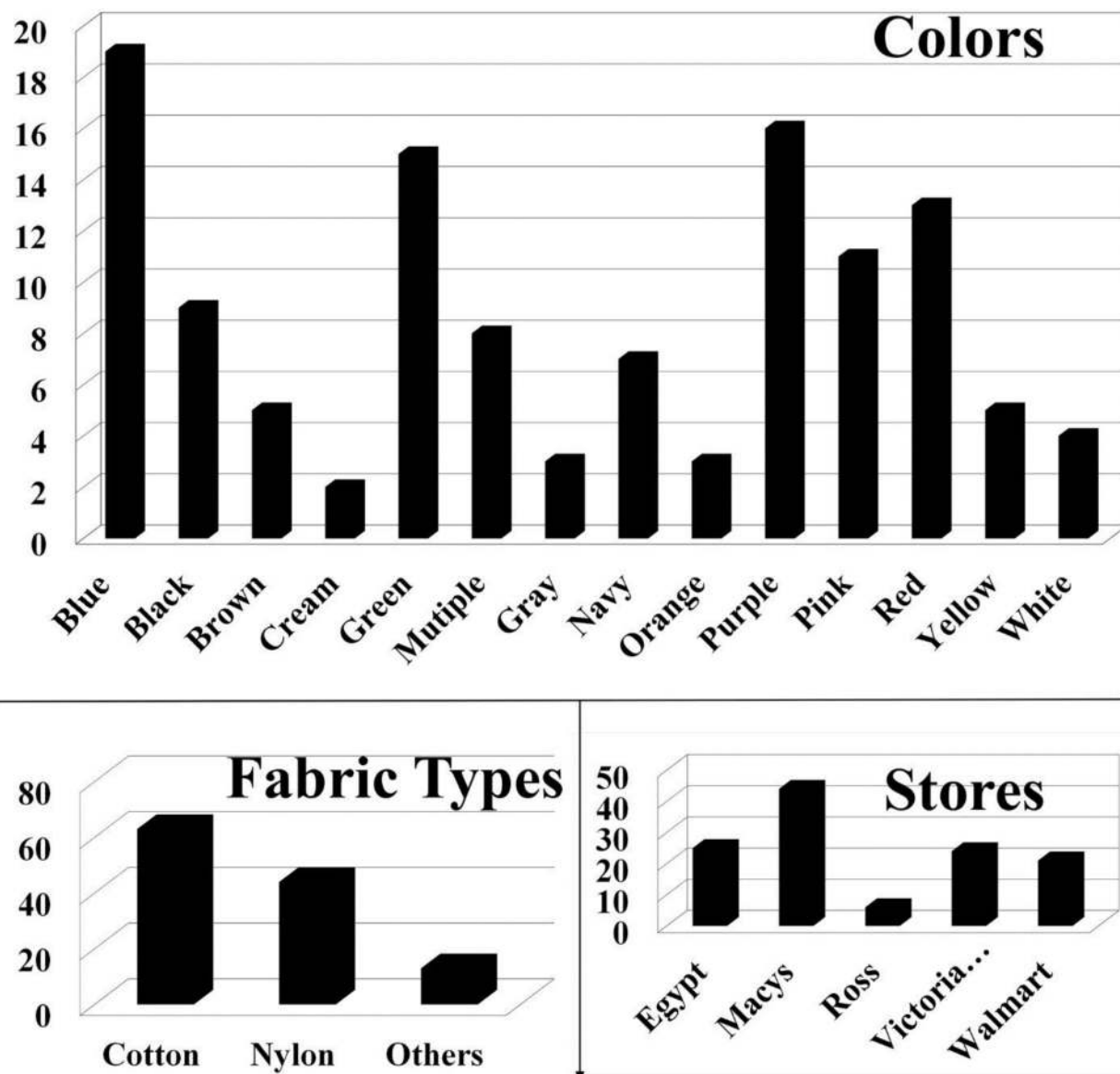
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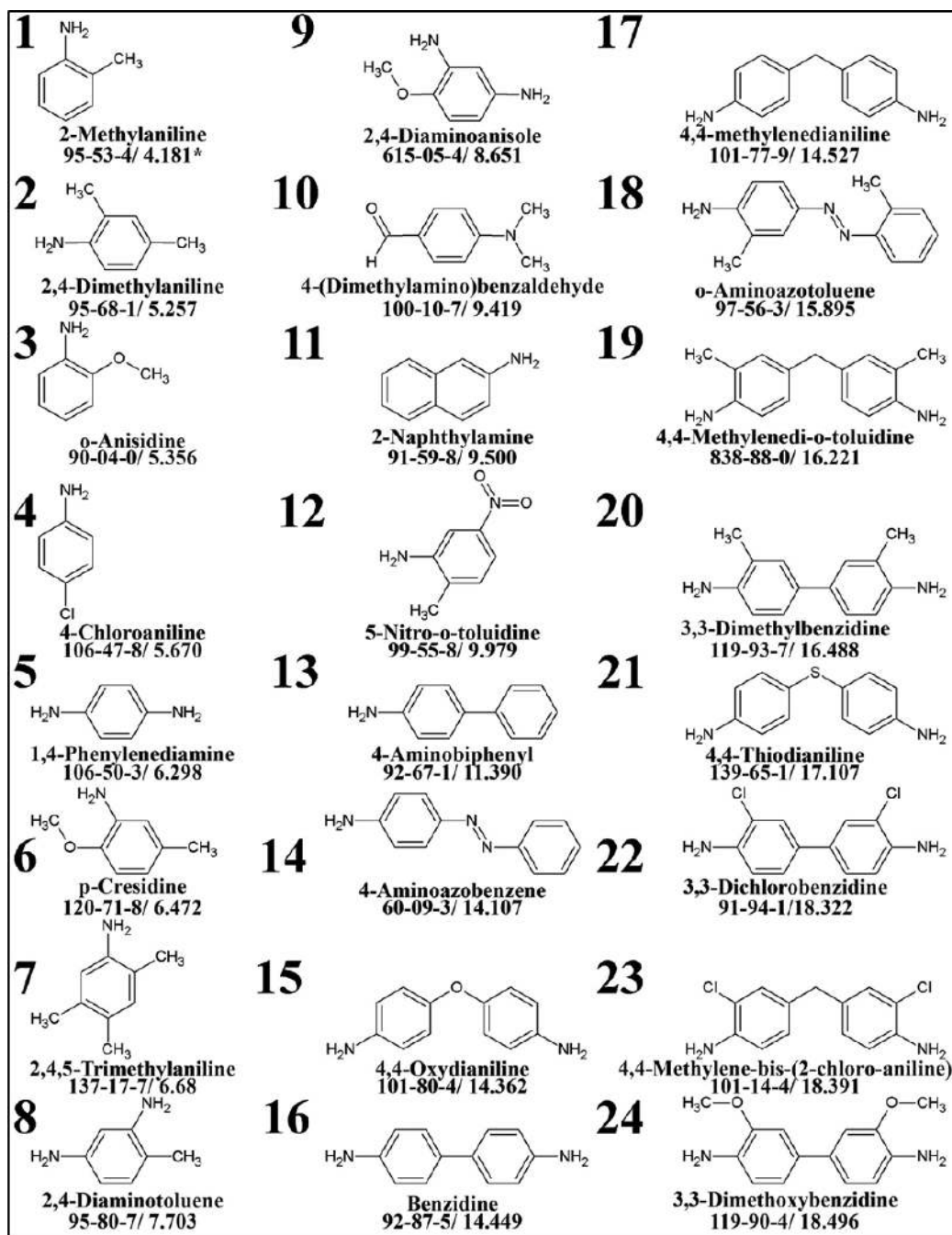
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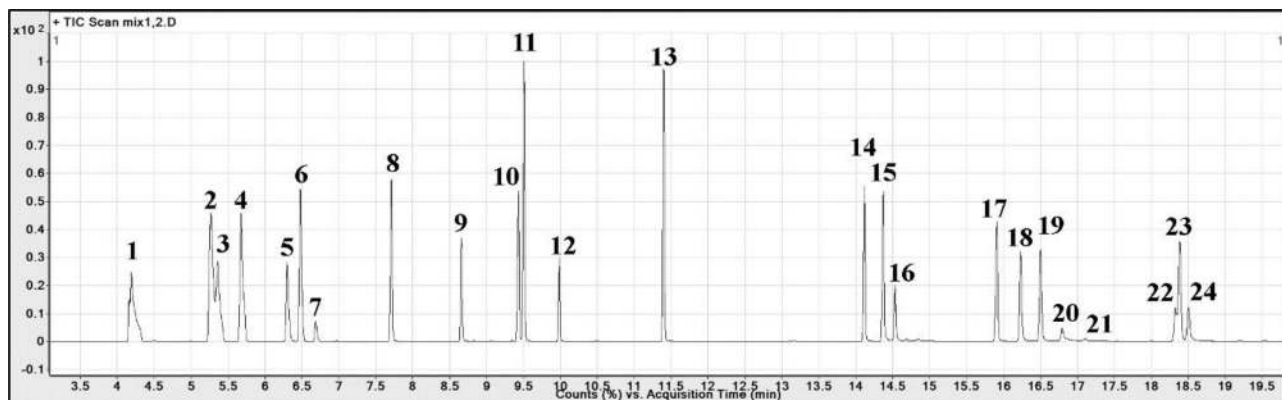




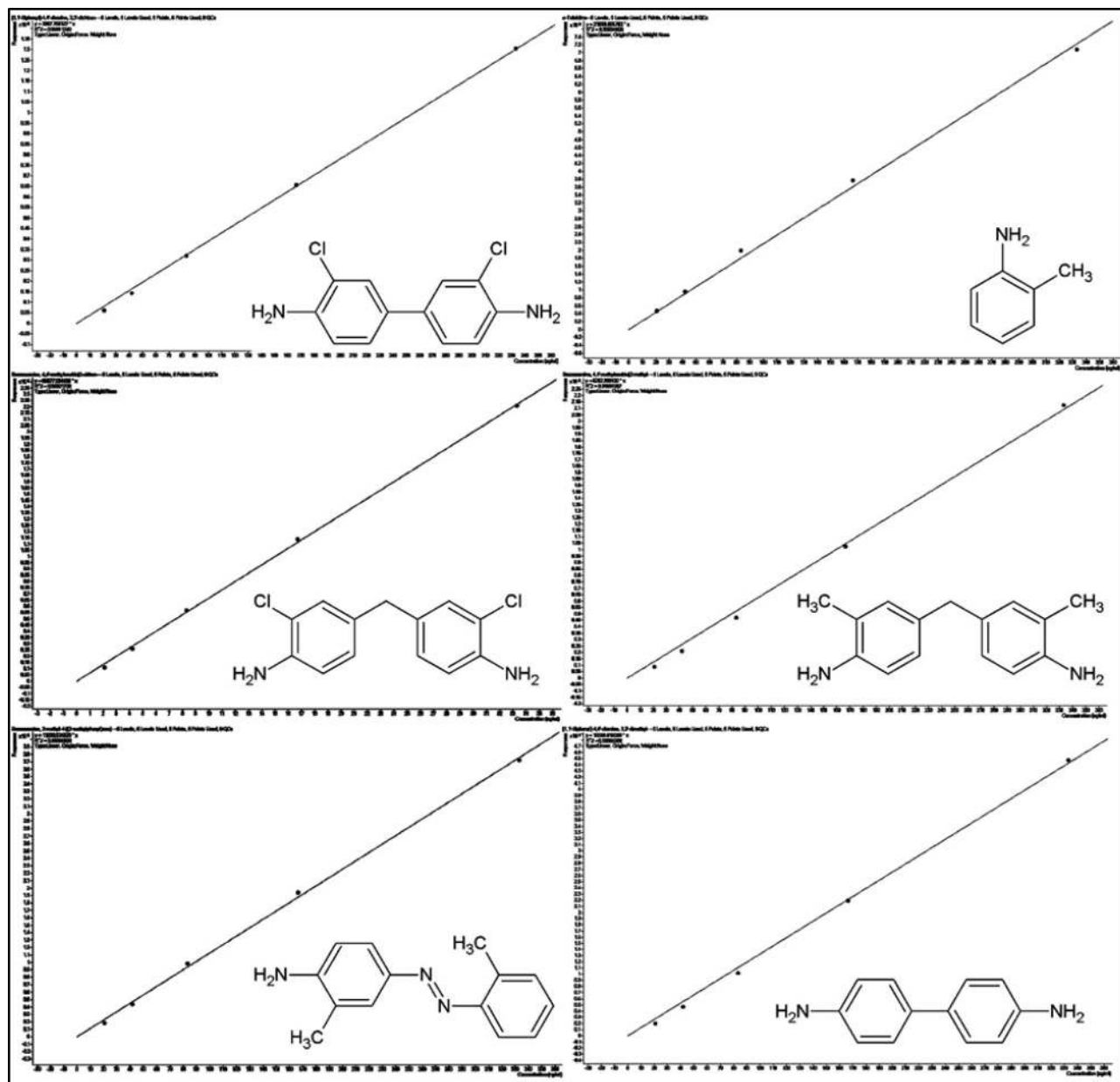
**Figure 1.**  
Samples collected according to their color, origin, brand names, locations and fabric types.



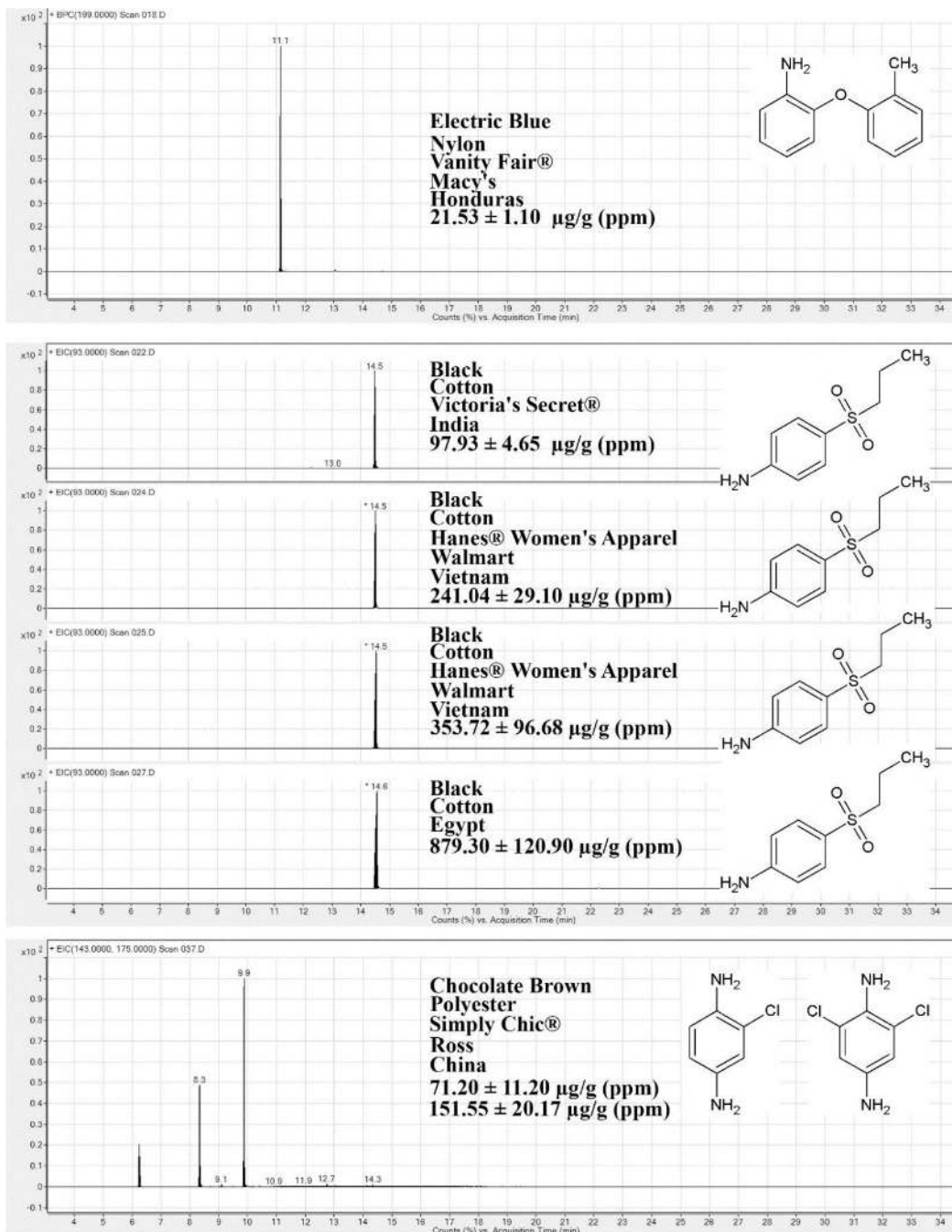
**Figure 2.**  
Chemical structure of the standard aromatic amines, their names, CAS# and GC retention times.



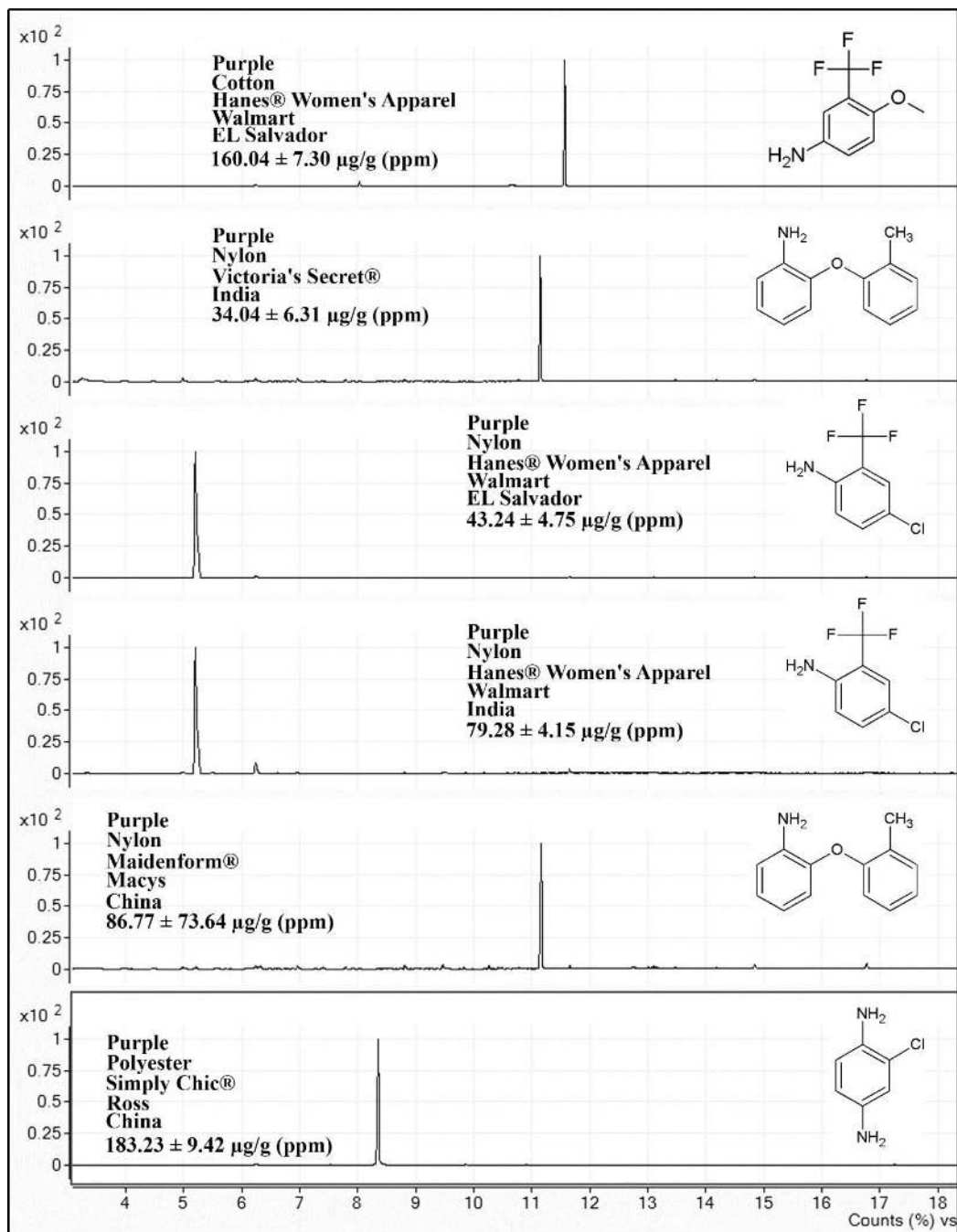
**Figure 3.**  
Total ion chromatogram of the 24 standard aromatic amines mixture.

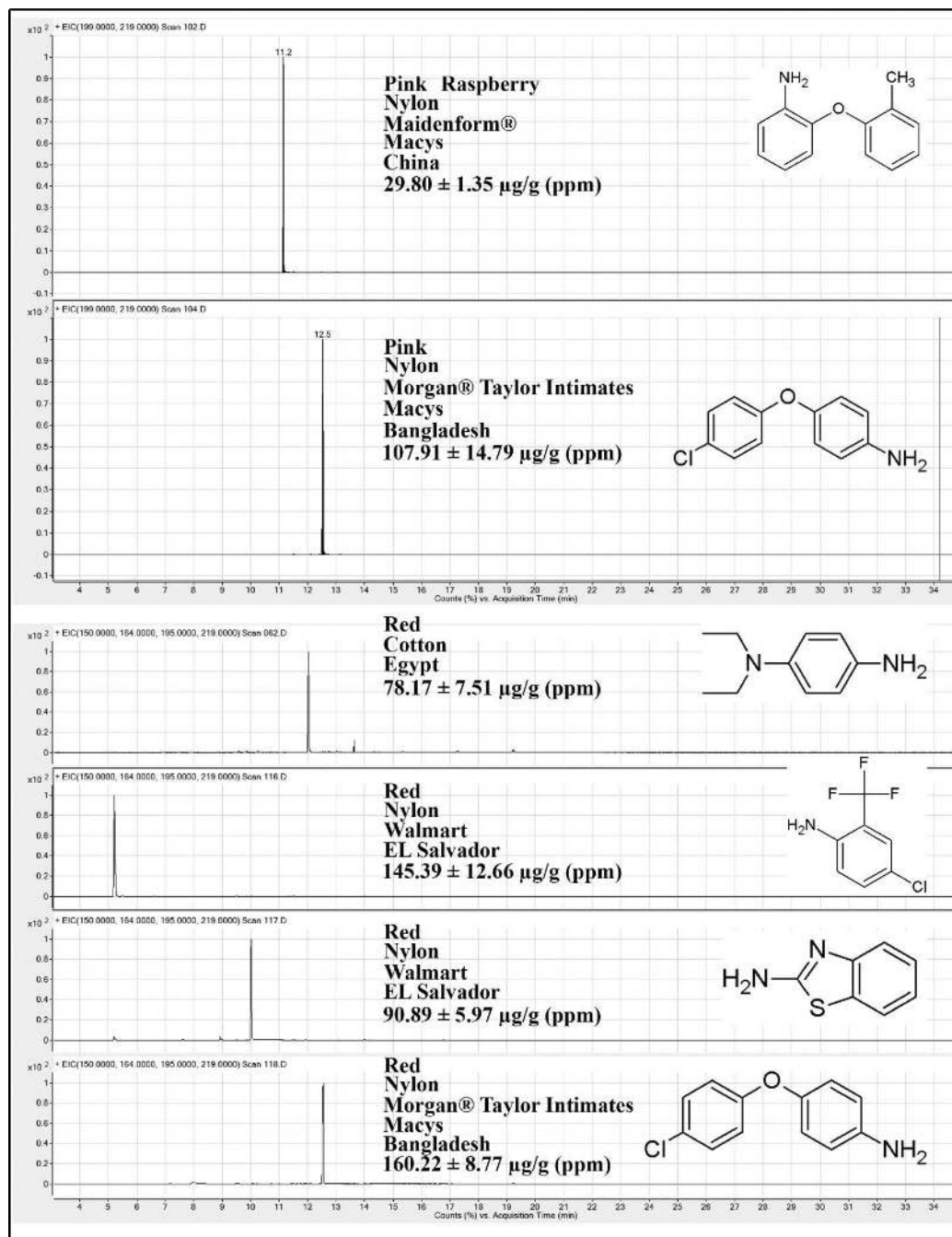


**Figure 4.**  
Calibration curves for selected amines showing detector response verses concentrations.

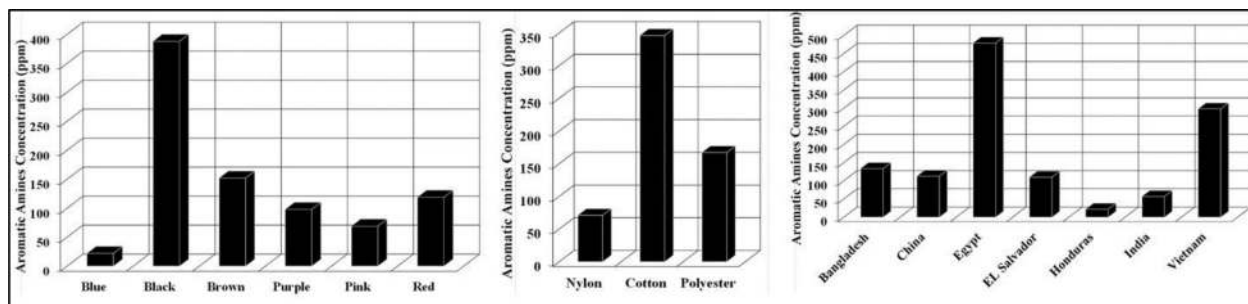




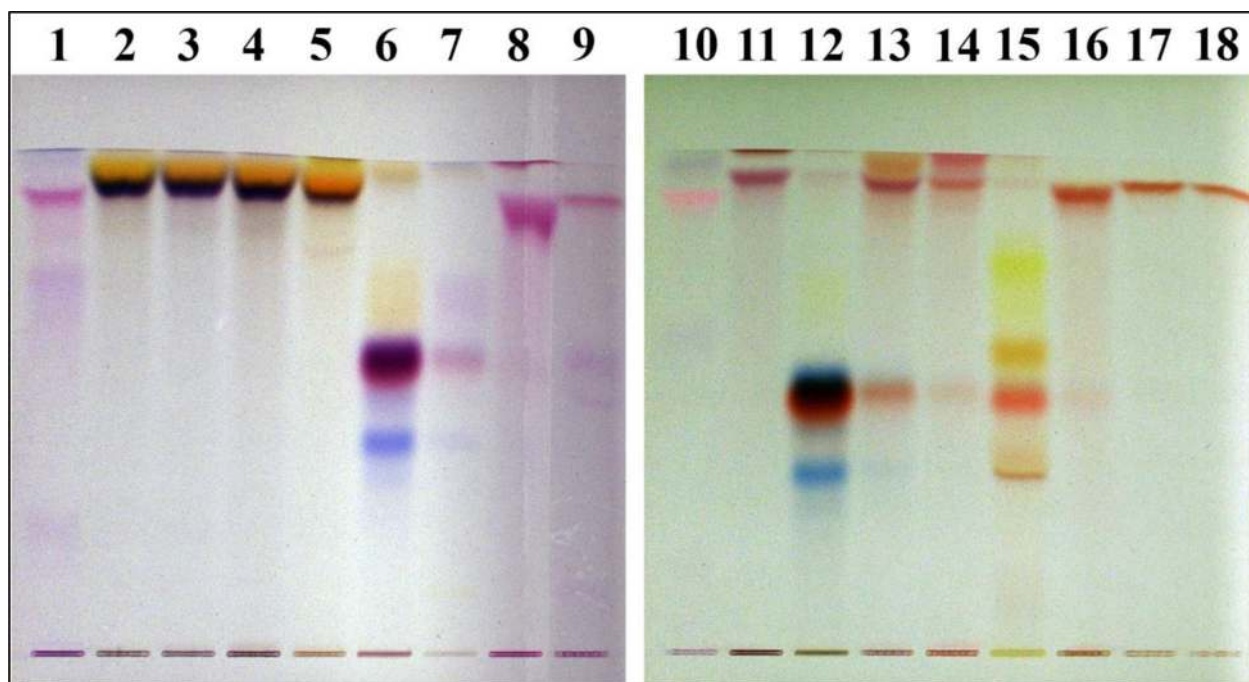


**Figure 5.**

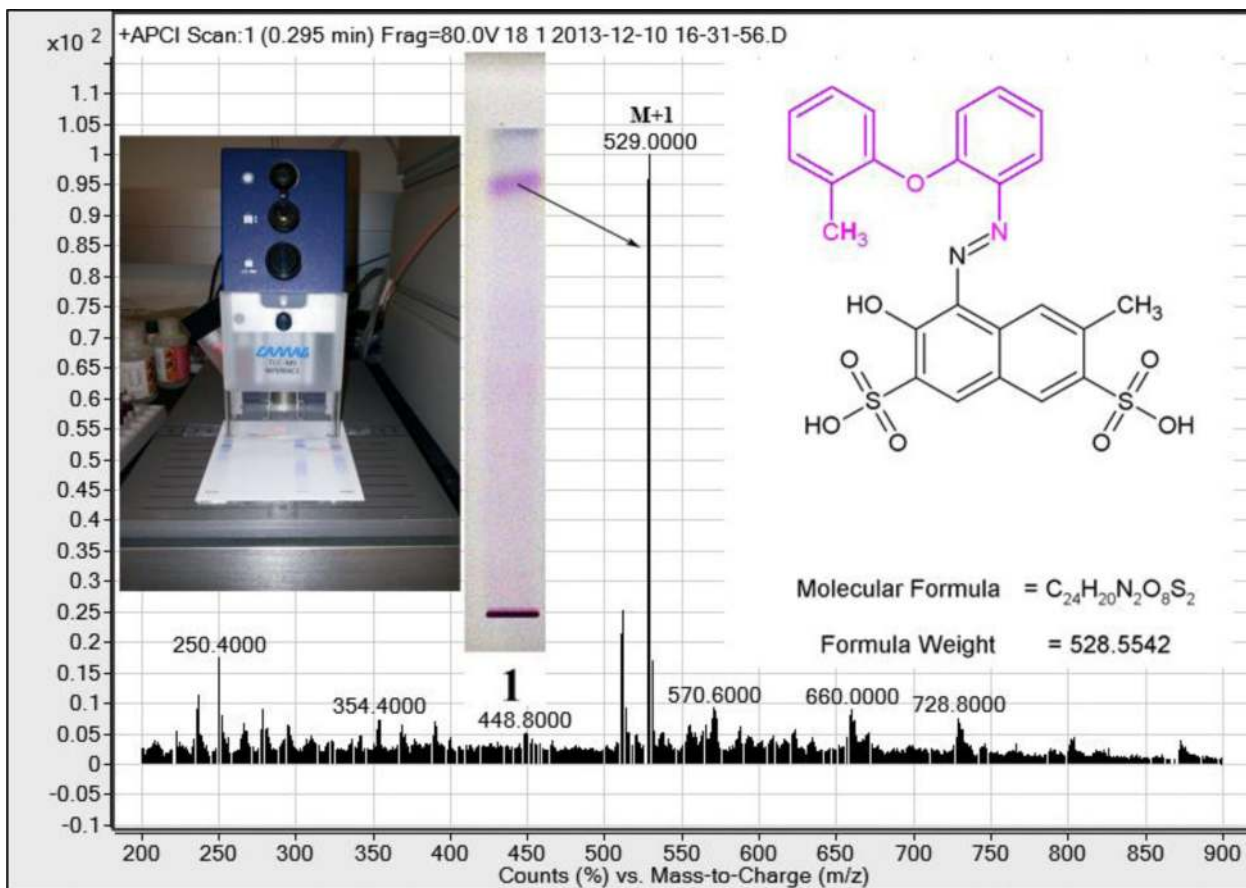
Total ion chromatograms of all of the 18 samples along with their color, brand names, sources, country of manufacturing as well as the identified aromatic amines and their concentration expressed as the average of three replicates and their standard deviation.



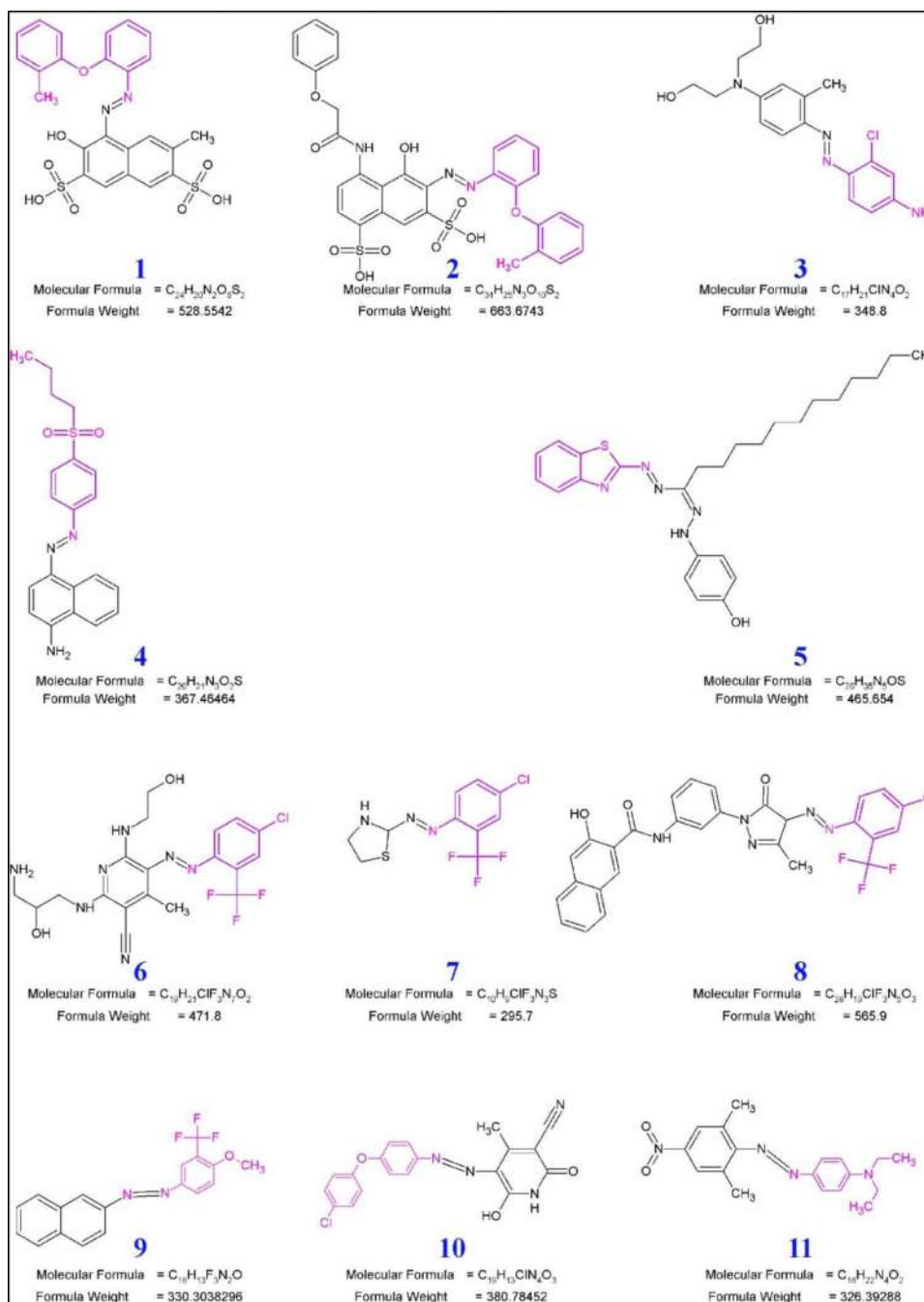
**Figure 6.**  
Aromatic amines concentrations according to samples color, country of manufacturing and fabrics type.



**Figure 7.**  
HPTLC visible images of the 18 selected samples azo dyes.



**Figure 8.**  
HPTLC-MS interface for direct mass spectrometry analysis of TLC bands.



**Figure 9.**  
Azo dyes identified