Screening of Some Banned Aromatic Amines in Textile Products from Indian Bandhani and Gamthi Fabric and in Human Sweat Using Micellar Liquid Chromatography

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Abstract

Certain dyes in textile products, which are capable of reductively splitting into carcinogenic aromatic amines, are strictly controlled in many countries. A simple, rapid, sensitive and green chromatographic method has been developed and validated for the simultaneous determination of 4-aminophenol (4-AMP), p-phenylenediamine (p-PPD) and benzidine (BNZ), banned aromatic amines in dyeing clothes and human sweat. The separation was achieved using a micellar mobile phase of 0.1M SDS, 4% 1- butanol (v/v) buffered to pH 7 with sodium dihydrogen phosphate, flowing under isocratic mode at 1 mL/min through a C_{18} column. Photodiode array detector was set at 210 nm. Using the above chromatographic conditions, 4-AMP, p-PPD and BNZ were eluted at 3.5 min, 4.7 min. and 5.4 min., respectively, adequately resolved. The method was validated by Standard Practices for Method Validation in Forensic Toxicology guideline for the industry in terms of selectivity, calibration curve, linearity (r^2= 0.999), trueness (relative bias, -3.5 to 7%) precision (relative standard deviation, <8.5%), and robustness of selected compounds. This method was sensitive enough for the routine analysis of aromatic amine in textile material with limit of detection in the (0.01 to 0.04 μg/mL range and limit of quantification (0.03-0.13 μg/mL). The method was successfully applied to dyed clothes and sweat samples. The main advantage of the developed method is the easy extraction step of the analytes from incurred samples without any further cleanup, which reduces per sample analysis cost and the total time of analysis. The developed method could easily replace regular chromatographic technique used for their detection.

Keywords: Carcinogenic; Chromatography; Dyes; Sweat; Textile; Validation.

1. Introduction

India is famous and well known for its different traditional and hand coloured fabric. Bandhani and gamthi are consumer choice nationally as well as internationally. Bandhani textile printing is a Indian traditional form of tie and dye which is about 5000 years old. Bandhni fabric reign supreme in Rajasthan and Gujarat which are home to an astounding variety of traditional craft. The beauty of bandhani fabric is that the fabric to be dyed is tied very tightly at different

points in the form of knots and then died with different colours. When this tight cloth goes for dying it doesn't catch colours and stay white or whatever colour the cloth has. Dyes used are natural and the background colours chosen are bright such as green, red, pink, yellow, black, among others Traditionally the fabric was died using madar root and pomegranate but as time passes different synthetic dyes are used to colour these fabrics. These synthetic dyes have harmful effects on human as well as aquatic animals. The addition of synthetic dyes deteriorates quality of traditional textile in National as well international market [1,2].

With the continuous change in the fashion trend and design, new textile material as well as new combination of colours are introduced in the market on regular basis. In developing and under developed countries, in order to reduce the cost of the manufacturing cheap colours are used in textile products. The dyes used to colour these traditional fabric sometimes contain cheap dyes. A lot of these cheap dye contains hazardous aromatic amines [3,4]. Aromatic amines are one of the noblest synthesis in the field of chemistry. Generally, they are used as an intermediate or precursor compound in various industries [5]. Since its discovery lots of aromatic amine based compounds like drugs, rubber, pesticides and azo dyes came into market [6]. Azo dyes belong to a group of organic compounds with an azo functional group in the molecule (-N=N). Azo colorants are the most commonly used group of dyes in textile industry and accounts for almost 60-70% of all dyestuff used in this industry [7,8]. Despite its huge consumption in textile industry, concern about the potential risk of azo dyes has been posed in past few decades. The risk factor is associated since in certain conditions, some azo dyes are cleaved by skin bacteria or by systematic metabolism what helps to release aromatic amines in several biological fluids (intestinal bacteria microflora, liver cells, extra hepatic tissue, epidermal cells and sweat), giving rise to undesired disorders like allergy and/or cancer [4,8,9]. Due to increased awareness about the harmful effect of azo dyes, government organization all over the world have drafted some regulations which specify the nature of these dyes and categorize them as permitted and non-permitted dyes. There are several regulatory bodies and government organization i.e. European Union Directive 2002/61/EC (Commission 2002) [10], Ministry of Environment and Forests, Government of India 1993 [11], German Consumer Goods Ordinance 1999, and France draft 1997, who have drafted regulations and specified the permitted dyes and their permissible limit with analytical methods to be opted in case of evaluating a product before its launching in the market. Some of the specific methods mentioned by German Institute of Standardization (DIN), International Organization for

Standardization (ISO) and Europäische Norm (EN); German DIN 53316, EN 14362-1:2003, EN 14362-2:2003 and ISO/TS 17234:2003 [12] are followed by most of the laboratories worldwide.

The basic biochemistry related with banned dyes is directly related to sweat glands which are present all over the body. Sweat is secreted by human body in response to various factor. During this process any foreign substances which comes in direct contact with skin diffuse inside human body [13]. In general, dyes present in cloth are very much polar which get easily extracted from the cloth. The main route of exposure to these dyes and their degradation product is either through oral ingestion (particularly by babies sucking on toys and textile), and dermal absorption through sweat [4,8]. However, due to ease of availability and low cost, textile industries in unorganized sector of developing and under developed countries are still using these dyes. This, many a time attract legal action incurring huge loss to the manufacturer or if exported could face severe penal action including damage to the reputation of the nation globally. To control this at initial stage, government agencies routinely screen textile products coming to the market from unorganized sector [4,8,14].

Regulatory agencies from different countries have focused attention on azo dyes containing the aromatic amines benzidine (BNZ; log Po/w = 1.34 [15]; pKa of the deprotonated form=3.3; pKa of the monoprotonated form=4.3 [16]), 4-aminophenol (4-AMP; log Po/w = 0.04; pKa-protonated form = 5.5; pKa2 = 10.5 [15]) and p-phenylenediamine (p-PPD; log Po/w =-0.25; pKa of the protonated form= 6.2 [15]) (Figure 1). Benzidine has been classified as group I, in terms of carcinogenesis effect [6]. Several governments and international organizations have imposed restrictions to the use of benzidine-based azo colorants in textile products that can potentially come into direct contact with human skin. They have been banned in India since 1993 [3]. European Union (2002) and China (2003) prohibited the use of azo dyes releasing more than 30 and 20 mg/kg, respectively, of the aromatic amine to biological fluids [12]. From 1992, OEKO-TEX® Standard 100 and Eco Mark refuses its certification to clothes containing these azo dyes [14]. The aromatic amines 4-AMP and p-PPD, although less toxic, can bring about some disorders [15], and then their use in textile has to be strictly controlled.

Many researchers have developed useful analytical methods for the screening of aromatic amines from azo dyes on separative techniques, such as high performance liquid chromatography (HPLC) [17-19], liquid chromatography coupled to mass spectrometry (LC-MS) [20-23], gas chromatography (GC) [24], gas chromatography coupled to mass spectrometer (GC-MS)

[8,14,25,26], High Performance Thin Layer Chromatography (HPTLC) [8], and capillary electrophoresis (CE) [27], in several matrices, like textiles [8,14,17,20,23-25], water [27], urine [26], blood [21], cell lysates [18], adhesives [22] and pure azo dyes [19]. As far as we know, no method about the determination of azo dyes in natural sweat has been released.

Although LC-MS is the technique-of-choice due to its analytical performances, it is an expensive technique (considering acquisition and maintenance), and requires large and laborious clean-up sample treatments. Therefore, the current trend in Analytical Chemistry consist in the development of easy-to-handle, cost-effective and eco-friendly alternative procedures [28]. Micellar liquid chromatography fulfills this requirement, and has been already used to determine aromatic amines used as primary intermediates and precursors used in the manufacture of azo dyes in waste water [29] and foodstuff [30].

The aim of the paper is the development of an easy-to-sample, cost effective, reliable and eco-friendly analytical procedure based on micellar liquid chromatography to determine the aromatic amines 4- aminophenol, p-phenylenediamine and benzidine (which may be present in azo colorants), in textile products and sweat. This would be useful to evaluate the risk to the consumer by the exposure to clothes containing azo colorants based on these aromatic amines. Procedure ought to be validated following an official guideline [31] and will be applied to incurred samples collected from several areas in India. The method should replace the current HPLC-based ones, and offer similar analytical performances at a lower cost and environmental impact.

2. Material and methods

2.1 Materials and Reagents

Solid analytical standards of benzidine (BNZ) with a purity ≥ 98.0%, 4-aminophenol (4-AMP) ≥ 98.0%, p- phenylenediamine (p-PPD) 98%, sodium dodecyl sulphate (SDS) ≥ 99.0%, and sodium dihydrogen phosphate (NaH_2PO_4) were procured from Sigma Aldrich (Mumbai, India). All the reagent used were of analytical grade and the solvents were of HPLC grade. Ultrapure Type-I obtained using Indion LAB Q Ultra system (Mumbai, India) was used throughout the work. Organic solvents used were 1-propanol, 1-pentanol procured from Spectrochem Pvt. Ltd.,

(Mumbai, India), 1-butanol and methanol were purchased from Central Drug House (New Delhi, India). For adjusting pH of mobile phase hydrochloric acid (HCl) 36.5-38.0% and sodium hydroxide (NaOH) were obtained from Sigma Aldrich (Mumbai, India) and Merck (Delhi, India) respectively.

An analytical balance Mettler-Toledo ME204 (Pocklington, United Kingdom) was used to weigh the standards and incurred samples. Magnetic stirrer and the ultrasonic bath (power: 230 V; frequency, 50 Hz) were purchased from PCI Analytics (Mumbai, India). The pH measurements were performed using a Contech LAB pH meter, Model pH-103 (Mumbai, India) equipped with a combined Ag/AgCl/glass electrode.

2.2 Preparation of solutions

Stock solutions (100 mg/L) of 4-AMP, p-PPD, BNZ each were prepared by weighing the proper mass and solving in ultrapure water, by hand-shaking and ultrasonication. Working solutions were prepared by successive dilutions. All these solutions were kept in amber vials at +4^{o}C.

Micellar solutions were prepared by weighing and dissolving the adequate amount of solid SDS and NaH_2PO_4 in Type-I water. Subsequently, pH was adjusted to the desired value by adding drops of either HCl or NaOH. Appropriate amount of short chain alcohol was added as modifier and Type-I water was added up to the mark in the volumetric flask. In order to have proper mixing of aqueous phase and organic modifier the mobile phase was sonicated for 5 minutes and filtered through 0.45 μm nylon membrane filter, with the aid of a vacuum pump.

2.3 Chromatographic equipment and operational conditions

Chromatographic analyses to detect and separate 4-AMP, p-PPD and BNZ in textile and sweat samples were carried out using a Shimadzu Prominence HPLC (Kyoto, Japan), equipped with a quaternary pump, an autosampler, and a Diode array detector SPD-M20A, connected to a PC. LC Solution software version 1.22 SP1 was used to control the instrumentation, as well as to register and process the chromatograms. Column was a C_{18} of 100 mm length, 4.6 mm internal

diameter and 5μm particle size, from Princeton Chromatography INC (Cranbury, NJ, USA). The mobile phase was an aqueous solution of 0.1M SDS -4% n-butanol, NaH_2PO_4 0.01 M buffered at pH 7, and run at 1 mL/min under isocratic mode. Injection volume and detection wavelength were 20 μL and 210 nm, respectively. All the analyzed solutions were filtered through a 0.45-μm Nylon membrane filter (Micron Separations) with a 3 mL syringe, and then introduced in the chromatographic vial. The specific HPLC use, care and cleaning instruction to-be-considered when dealing with micellar solutions can be seen in [32]. Following these instructions, the column may reach a lifespan of more than 1000 injections.

The experimental dead time (t0) was ≈ 1.5 min. The retention factor (k'), the efficiency (N) and the asymmetry (T) were calculated as in [33].

2.4 Sample collection and preparation

2.4.1 Textile

In order to detect the presence of selected aromatic amines in fabric, ninety textile samples (30 from each city) of different colors were randomly collected from Sagar (Madhya Pradesh), Ahmedabad (Gujrat) and Jaipur (Rajasthan). The sample collection strategy was categorized in three main shades (dark, intermediate and light) used as colorant in textile material. 30 samples which included 10 color from each group were collected from each city. Selection of cities for the collection of samples was based on their known popularity in handprint as well as their importance in textile manufacturing. The two neighboring state of Madhya Pradesh (the state of Gujrat and Rajasthan) are hub of bandhani and gamthi print which are in great demanded in India as well as in international market. Since, the work was carried out in Sagar (Madhya Pradesh) so textile samples were also collected from this city.

The textile material accurately measured 10×10 cm was placed in beaker and soaked in 5 mL of Type-I water for 30 min followed by ultra-sonication for 15 min. After sonication the supernatant was collected and filtered through 0.45 μ nylon membrane filter for injection. The glass bottles containing cotton pads were sonicated for 15 min. and after that the cotton balls were squeezed using forceps. The obtained extract was filtered through 0.45μm nylon membrane filter and directly injected on to chromatographic column.

2.3.2 Sweat Sample

Sweat was taken by passive sampling without sweat induction. The volunteer study was carried out under the ethical number of the project F.No.1012/CST/R&D/Phy.Engg.Sc/2015. A piece of textile material (10×10 cm), which shows positive for BNZ, was put in the form of scarf around the lower mid-range of the neck. After one hour the scarf was removed and the neck area covered by scarf was wiped with the help of cotton pad, which was further squeezed up to the obtaining of 1 mL of sweat. The similar procedure was followed five times using different cotton pads and placed in clean glass bottles [34]. Five research group members who consented as volunteer, wore new stitched cloths and sweat sample were collected form these volunteers also. The procedure was followed by five volunteers of research group and a total 5 samples were obtained.

2.5 Method validation

The proposed method was validated following the Standard Practices for Method Validation in Forensic Toxicology guideline [31]. Evaluated analytical performance parameters were: linearity, selectivity, precision, trueness, limit of detection, limit of quantification and robustness. The statistical calculations were performed as indicated in [33,35,36].

2.5.1 Linearity and sensitivity

Seven standard solutions containing increasing concentrations of the aromatic amines (up to 10 mg/L) were analyzed by pentaplicate. The lower and upper variance were compared by a Snedecor's F-test (significance level of 5%) to evaluate homocedasticity.

The slope, y-intercept and determination coefficient (r^2) were obtained by plotting the average peak area v.s. the corresponding concentration by least square linear regression. The goodness-of-fit was appraised by the r^2, relative residual standard deviation (RRSD) and distribution of residuals v.s. concentration. The signal of the blank was evaluated through the y-intercept. Levels with a relative residual higher than 3 were considered outliers.

The limit of detection (LOD) was calculated as 3.3 times the standard deviation of the blank (standard deviation of the y-intercept) divided by the sensitivity. The limit of quantification was the lowest concentration fitting the acceptance criteria for bias and precision (<20%).

2.5.2 Precision and trueness

Intraday values were determined at five levels over the linear range by the analysis of blank samples of textile and sweat by sextuplicate. Trueness (relative bias) was the difference between the average found concentration minus the true value, divided by the true value, while the precision (repeatability, as relative standard deviation) was the relative standard deviation of the six found concentrations.

To calculate the interday values, the same procedure was carried out by different operators using renewed solutions over a 3-month period. Trueness was the difference between the mean of all the obtained values minus the true value, divided by the true value. Precision (intermediate precision) was the relative standard deviation of the obtained values.

2.5.3 Robustness

The robustness of the developed method was studied by generating small variations in the constituent of the mobile phase and evaluating the subsequent variation on the chromatographic responses (retention time and peak area). In this study the changes were as follows: SDS concentration (0.09-0.11 M), 1-butanol (3.5%-4.5%), pH (6.5-7.5), and flow rate (0.9–1.1 mL/min). The effect of each factor was examined one-by-one. A solution containing the three studied compounds (at a concentration of 5 mg/L) was analyzed at the low, average and high level of the studied factor, while the others remain constant. The RSD of the responses was used to appraise robustness against the oscillation of the modified factor. This procedure was carried out for each above indicated experimental condition.

3. Result and discussion

3.1 Method development

Method optimization in MLC is similar to that of HPLC, which normally starts with studying the physiochemical property of the selected analyte. The major difference between MLC and HPLC is the use of surfactant at a concentration above the CMC (Critical Micellar Concentration) of the surfactant together with a short chain alcohol, which acts as a modifier. The concentration of the modifier does not exceed 10% v/v. thus, making it a preferred liquid chromatographic technique best suited for qualitative and quantitative analysis. Based on the previous research work using MLC, SDS and C_{18}-column were selected as the surfactant and column of choice [37]. The other important parameters considered for optimization of mobile phase are discussed below.

3.1.1 Selection of wavelength for absorbance detection

Aromatic amines are group of organic compounds, which show strong absorbance in UV region mainly due to presence of conjugation and lone pair of electron. 4-AMP, p-PPD and BNZ also behave in the same manner and show good absorbance in UV region. In the present work a simple UV-visible spectrophotometer was used to find out the wavelength of maximum absorbance for these aromatic amines. All the three compounds showed maximum absorbance at 210 nm, however 4-AMP showed two more absorption maxima at 283 nm and 312 nm which were almost 10 and 5 times less than that of the absorption obtained at 210 nm. Similarly, p-PPD and BNZ also showed one more absorption maxima at 240 nm and 285 nm respectively which was again 5 times and 4 times lower than that obtained at 210 nm. In the present case, a blank sample was analyzed at 210 nm, which showed no significant absorption. Thus, it was decided to carry out further analysis at 210 nm.

3.1.2 Selection of the pH of the mobile phase

The considered pHs were 3; 5 and 7; all of them inside the working pH of the column (2.5 – 7.5) [38]. While optimizing this parameter, the protonation constant (pKa) of the soluted should be considered, given that it provides an idea about the charge of the molecule at different pH

values. According to their structure, the three compounds contain only one sort of ionizable group, NH_2, which can be protonated (NH_3^+) or neutral (NH_2).

The lower pH, the higher positive charge and the more expected retention, due to the interaction of the cations with the negative layer of the stationary phase [38]. At pH 7, the three compounds are neutral. The experimental retention times obtained for 4-AMP, p-PPD and BNZ at pH 3 was above 15 min, which drops significantly to 6 min. at pH 7. Thus pH 7 was finally selected for analysis of selected aromatic amines using MLC.

3.1.3 Optimization of SDS concentration and organic modifier

SDS was selected as preferred surfactant for the separation of selected aromatic amines. For enhancing the chromatographic parameters such as efficiency (N), asymmetry factor (B/A) and retention factor (k') it is always recommended to add a small amount of short chain alcohol like 1-propanol, 1-butanol and 1-pentanol. As the analytes are moderately polar and neutral, the mobile phases using 1-pentanol would exhibit a too strong elution power, and then was discarded. 1-butanol was preferred over 1-propanol, as the first one would improve the efficiency of the peaks.

In order to study the chromatographic behavior of the aromatic amines, they were analyzed at five mobile phases (SDS, M/1-butanol, %, v/v): 0.05/1; 0.05/7; 0.15/1; 0.15/7; and 0.1/4 [38]. The obtained chromatographic parameters (k', N and B/A) can be seen in Figure 2. The maximum resolution at the minimum analysis time was reached at 0.1M SDS with 4% 1-butanol. This mobile phase also gave the maximum efficiency and the asymmetry closer to 1 for the three aromatic amines. Therefore, this mobile phase was considered as the most suitable for the analysis. Chromatograms obtained from the analysis of standard solutions of the aromatic amines can be seen in Figure 3. The obtained retention times (min) were BNZ, 3.5; p-PPD, 4.3; 4-AMP, 5.8.

3.2 Method validation

3.2.1 Linearity and sensitivity

Results of the linearity and sensitivity study can be seen in Table 1. The method was proven homoscedastic inside the linear interval. Goodness-of-fit was adequate, as r^2 >0.999, RRSD < 1.5% and the residuals were randomly distributed around zero. Signal of the blank was null, as the y-intercept confidence interval includes 0; and no outliers were noticed.

Sensitivity is largely enough to detect the aromatic amines, released by the azo dyes, in the extracts and biological fluids at the maximum permitted level stated by Chinese (20 mg/L) and EU (30 mg/L) regulation [12].

Table 1. Analytical performance of the proposed method (concentrations in µg/mL)

Analyte	Regression equation			Linear range	Relative residual standard deviation%	LOD	LOQ
	Slope	Intercept	Determination coefficient/r^2				
BNZ	6.223±0.008	0.058±0.008	0.999	0.15-50	0.81	0.09	0.27
4-AMP	2.162±0.005	0.534±0.006	0.999	0.05-50	0.42	0.08	0.26
p-PPD	5.23±0.04	-0.73±0.19	0.999	0.15-50	0.60	0.02	0.07

3.2.2 Selectivity

Blank matrix of textile and sweat were analyzed to examine the selectivity. Both chromatograms displayed several peaks at the front, corresponding to other colorants and salts and biological compounds excreted from out body. However, those eluted before the first aromatic amine. No peaks were observed at the window time of the aromatic amines, thus ensuring the absence of interfering compounds.

During analysis of incurred samples, no interference from the matrix was observed. In order to further confirm the matrix effect, both matrices were spiked with 4-AMP, p-PPD, BNZ and chromatographed (Figure 4A). The chromatogram obtained for the three analytes did not show any interference from the matrix.

3.2.3 Precision and trueness

Results of precision and trueness study are in Table 2. The data shows good trueness (-3.5 – 7.0%) and adequate precision (<8.4%), under the requirements of the guideline (<20%), and then proving the reliability of the method.

Table 2. Intra[a]- and inter[b]-day trueness (%) and precision (%) for the studied aromatic amines

Aromatic Amine (matrix)	Spiked conc. (µg/mL)	Intra-day		Inter-day	
		Trueness	Precision	Trueness	Precision
BNZ (cloth)	0.2	2.5	2.4	6.5	3.3
	1	5.3	4.5	5.5	6.0
	2.5	1.3	0.8	3.6	1.6
	5	2.0	1.5	0.9	1.5
	10	0.6	0.9	0.7	1.4
p-PPD (cloth)	0.2	-3.5	4.1	4.5	4.3
	1	1.7	5.4	5.1	6.7
	2.5	-0.3	1.4	2.6	2.7
	5	-0.2	1.7	3.0	2.4
	10	-0.5	2	1.8	2.4
4-AMP (cloth)	0.075	4.0	6.4	2.7	8.2
	1	2.7	8.4	3.1	7.3
	2.5	1.4	2.5	-2.5	2.8
	5	-0.4	2.8	1.0	4.2
	10	-0.8	1.5	-0.5	2.1
BNZ (sweat)	0.2	6.5	5.3	4.0	7.4
	1	6.0	4.8	7.0	7.5
	2.5	-2.8	2.3	-2.0	3.7
	5	3.0	1.8	2.6	2.8
	10	-1.9	1.4	-1.6	2.3

a n=6; b n=5

3.2.4 Robustness

The small experimental oscillations in the main chromatographic conditions that may happen during routine analysis have no significant influence in the retention time (RSD < 6.5%),

efficiency (RSD < 2.6%) and the asymmetry factor (RSD < 5.6%) for 4-AMP, p-PPD, BNZ. Therefore, the method is robust enough for the qualitative and quantitative analysis of selected aromatic amines.

3.2.5 Advantages of the procedure

As far as we know, this is the first paper devoted to the determination of 4-AMP, p-PPD and BNZ in the sweat induced by wearing a dyed cloth (wherein is the hazard for human health lies), instead in the cloth itself.

Regarding the chromatographic step, the three compounds were simultaneously determined by HPLC-MS in [23]. As advantages of the here proposed method, we can highlight: the use of mobile phases with low amount of toxic and volatile solvent (4% 1-butanol; 50% methanol [23]), the possibility to resolve 4-AMP and p-PPD (they exhibited almost the same MS-transitions with same retention time [23]), slightly higher linearity (r^2> 0.999 to 0.997 [23]), precision (RSD <8.4% to 9.5% [23]) and trueness (-3.5 – 7.0% to -10.5 - 11.6% [23]) and duration of the chromatographic run (more retained aromatic amine 5.9 min to 7.2 min [23]), as well as the cost of the analysis (the use of mass spectrometry strongly increase the cost). As disadvantage, we have to admit the HPLC-MS-method exhibits higher sensitivity (LOQ in solution, 0.01 – 0.025 mg/L [23] to 0.02-0.09 mg/L) and selectivity, as well as the possibility to simultaneously determine other aromatic amines. However, these drawbacks do not impair the development of the study. We stress out similar analytical performances were reached using far less economic resources, and with less environmental impact.

3.3 Application to incurred samples

The textile samples collected from non-branded textile shop were analyzed in the present study to detect the presence of 4-AMP, p-PPD and BNZ. As mentioned earlier a major portion of Indian textile industry is fall in unorganized sector where no stringent regulations are followed so there is a possibility of use of cheap banned dyes during manufacturing and finishing. In this study textile material collected from Jaipur (2.4.1) showed the presence of BNZ in four moderate coloured textile sample and 4-AMP in eight dark and moderate coloured textile samples out of

which the highest found concentration were for BNZ and 4-AMP (0.61 μg/mL and 0.87 μg/mL respectively). In the same study the lowest concentration obtained were 0.15 μg/mL and 0.18 μg/mL respectively for both of the above mentioned analytes. Apart from this, in some of textile material the studied aromatic amines were not detected either due to their absence or due to the possibility of falling in low concentration range than the detection limit of these analytes. Samples obtained from Gujrat also showed the same trend. In these textile sample out of 30, eleven shows positive result for 4-AMP and six for BNZ. When textile samples obtained from Sagar were analyzed, one of them showed very high concentration of 4-AMP (335.1 μg/mL) and p-PPD (18.9 μg/mL), which was a dark colored cloth (Figure 4B). Here also in dark and intermediate color cloths showed the presence of 4-AMP and P-PPD whereas BNZ was negative in dark color clothes but was detected in six intermediate colored textile (Figure 4C).

The general trend, which was observed during textile sample examination, was that in dark and intermediate colored cloths 4-AMP, p-PPD were present. Whereas BNZ was only detected in intermediate colored textile sample collected from the selected cities. In light colored clothes results for all the three compounds were negative.

After analyzing all the textile samples, it may be concluded that 48 sample were positive for selected aromatic amines, in which some of them were showed the presence of 4-AMP, p-PPD and BNZ individually or in combination in dyed textile. However, all three aromatic amines were not detected in any of the sample. The most frequently found aromatic amine was 4-AMP (28), BNZ (16) and p-PPD (4), as displayed in Figure 5.

Once the presence of these three compounds were confirmed in the textile sample the textile sample containing BNZ were tailored into scarf and given to volunteers as mentioned in section 2.4.2. In this only BNZ containing textile sample were tested for dermal exposure because this is carcinogenic and may cause toxic and harmful effects once it is inside human body. The common local method of colouring is to dip dyed cloth in salt water for colour fastening. The basic path of entry of banned dyes in human body is skin as sweat gland are present all over the body. The salt acts as an ionic carrier for the dyes as the dyes become polar once they are dissolved in salt.

The sweat also contains salts excreted from our body and therefore the sweat samples of the volunteers who wore cloth which contain BNZ, were directly injected on the chromatographic

instrument in the optimized chromatographic condition. Out of five samples, one was found positive and the obtained concentration was 0.7 μg/mL (Figure 4D).

4. Conclusions

The present work describes a reliable and suitable method based on MLC coupled to UV-Visible absorbance to detect 4-AMP, p-PPD and BNZ in cloths and sweat sample with an analysis time below 8 min. The method is unique as no work of such easy extraction of aromatic dyes from textile and sweat sample for HPLC or MLC determination has been developed before.

The procedure was validated using Standard Practices for Method Validation in Forensic Toxicology guideline for separation, detection and quantification of the above mentioned aromatic amines. The developed method was efficient with satisfactory results in term of selectivity, precision, trueness and robustness studies. The limits of detection and quantification were good enough to monitor these compound in the extract of textile and sweat sample, and levels under the maximum tolerable levels stated by the regulations were largely attained. The method employed here is very easy-to-conduct, economic and rapid as an easy pretreatment is required for the textile, and no one for sweat sample. Moreover, the developed method is green as it reduces drastically the organic solvents used in hydroorganic reverse phase (RP)-HPLC, and exhibits a high sample throughput. The method was applied to commercial textile and sweat sample, wherein some turned up to contain measurable amounts of 4-AMP, p-PPD and BNZ. Therefore, the RP-HPLC method used for the routine analysis of these dyes can be replaced with the MLC method developed here as it is very fast ecofriendly technique. In the future, we aim to apply the procedure to determine other aromatic amines in cloths elaborated with other azo-dyes and sweat, by varying the chromatographic conditions.

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6. Conflict of Interest

The authors declare no conflict of interest.

7. References

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FIGURE CAPTIONS

Figure 1. Absorbance spectrum and structure of the studied aromatic amines

Figure 2. Effect of different concentration of SDS/1-butanol on the chromatographic responses for p-PPD, BNZ, 4-AMP: A) capacity factor; B) efficiency (N) and C) asymmetry

Figure 3. Chromatograms obtained by the analysis of standard solution of 5 mg/L: A) BNZ; B) p-PPD and C) 4-AMP.

Figure 4. Chromatograms obtained by the analysis, under the optimal conditions, of textile samples: A) blank spiked at 0.5 mg/L 4-AMP; p-PPD and BNZ (absorbance measured at 302 nm): B) incurred SH1 (10x10cm Fabric), containing 335.1 μg/mL 4-AMP and 18.9 μg/mL p-PPD; C) incurred SE1, containing 2.4 mg/L, BNZ; and D) a sweat sample were 0.7 mg/L of BNZ was found.

Figure 5. Number of samples in which 4-AMP, p-PPD or BNZ were found.