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ARTICLES

Simultaneous Determination of Harmful Aromatic Amine Products of Azo Dyes by Gas Chromatography–Mass Spectrometry

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Abstract—Azo dyes constitute about 66% of all colorants and they are widely used in industries such as textiles, plastics, ceramics, cosmetics and food. However, due to the reductive cleavage of some azo dyes into carcinogenic aromatic amines, many countries have set concentration limits or have banned the use of these azo dyes. Selected aromatic amines that pose health risk to consumers were simultaneously determined by gas chromatography mass spectrometry. A good linearity in calibration plot was obtained for the analytes and the low relative standard deviations indicated high instrumental precision. A recovery test was performed to validate the extraction method and the results obtained were between 92–114%. The method was applied to the analysis of real samples and the concentrations of aromatic amines detected were below the 30 mg/kg limit set by regulatory authorities.

Keywords: azo dyes, aromatic amine, gas chromatography–mass spectrometry, reductive cleavage

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Colorants are substances that are added to alter the color of substances [1]. The term colorant is used to describe dyes and pigments which differ from each other based on their solubility in the application medium. Dyes are soluble in the application medium (particularly water) unlike pigments that are dispersed into a medium where they are mechanically attached to the substrate with the help of a dispersing agent [2]. Naturally, dyes are extracted from sources such as vegetables (plants, fruits, seeds) and animals (mollusks, insects), while pigments are obtained from coloured earth minerals [3]. Advancements in the production of synthetic dyes and pigments have broadened the scope of applications.

Picric acid, the first synthetic dye, was developed by Peter Woulfe in 1771, but it was commercially unsuccessful due to its poor light fastness property [4]. A more significant breakthrough occurred in 1856 when William Henry Perking accidentally produced the dye mauve in his attempt to produce the antimalarial drug quinine [5]. The pursuit of more chemists into the synthesis of synthetic dyes led to the discovery of azo dyes in 1858 by Peter Griess. In his work, nitrous fume was passed through picramic acid to produce a cationic product belonging to a new class of compounds [6]. Extending his work to primary aromatic amines led to the formation of diazo compounds. In the classification of colorants by chemical structure, azo dyes constitute about 66% of colorants and therefore have a wide range of applications [7].

Despite the numerous applications and benefits of azo dyes, some carcinogenic effects in humans have been reported. Under anaerobic conditions and in the presence of trace amount of sodium dithionate, azo dyes are reduced to two aromatic amines at 70°C [8, 9]. Also, it has been reported that some anaerobic microbial organisms break down azo dyes into colorless aromatic amines [10]. Some occupational exposures to 4-aminobiphenyl, aniline and benzidine in China and Germany led to bladder cancer among some workers [9, 11–14].

The German Consumer Goods Ordinance was thus amended in 1994 to ban azo dyes that are reduced to 20 aryl amines including benzidine and its disubstituted derivatives classified as carcinogens [15–17]. The ban came into force in 1996 and this was followed by other members of the European Union creating similar but different instructions stipulated by the MAK ("Maximale Arbeitsplatzkonzentration": maximum workplace concentration) commission. A mandatory amine concentration limit of 30 mg/kg was placed on finished products or in dyed parts of a product [18]. In 1995, dye products containing aromatic amines specified in the MAK III A1 list (direct threat

to human health) or MAK III A2 list (indirect threat to human health) were prohibited from import and usage in Turkey. Inspections are therefore made by sampling and analyzing dyed textiles to ensure compliance with the regulation [15].

Two standard methods are used for the determination of aromatic amines: EN 14362-1 and EN 14362-2, where the latter covers the extraction of fibre materials [19]. After the reductive cleavage of the azo group, methods such as solid phase extraction and diatomaceous earth filled columns have been used to extract aromatic amines from the aqueous solution [19, 20]. Chromatographic methods have been widely used in the separation and identification of aromatic amines based on properties such as polarity, vapor pressure and electrophoretic mobility. These include HPLC, thin layer chromatography, high performance thin layer chromatography, gas chromatography and capillary electrophoresis [9, 20–22].

The aim of this study was to develop a simple but efficient extraction method for selected aromatic amines and determine percent recoveries to validate the extraction method. The method was then applied to a variety of dyed products on the market and analyte determinations were made by gas chromatography–mass spectrometry (**GC–MS**).

EXPERIMENTAL

Reagents. All chemicals and reagents used were of analytical grade. A 50 mg/L mixed standard stock solution and working standards of the aromatic amines were prepared in acetonitrile obtained from Merck, Germany. The analytes included 4-chloro-*o*-toluidine (CAS: 95-69-2), 4-aminobiphenyl (CAS: 2243-47-2), 2,4,5-trimethylaniline (CAS: 137-17-7) and benzidine (CAS: 92-87-5). Sodium dithionate, *tert*-butyl methyl ether, trisodium citrate dihydrate, sodium hydroxide and hydrochloric acid used in the reductive cleavage and extraction process were also obtained from Merck. Ultrapure water with 18.2 MΩ cm resistivity was obtained from a Milli-Q® Reference System at the central laboratory of Yıldız Technical University.

Gas chromatography–mass spectrometry analysis. All measurements were done on an Agilent GC–MS (GC 6890 – MSD 5973) system fitted with an HP-5 capillary column (30 m × 0.32 mm I.D., 0.25 μm film thickness). Helium was used at a constant flow rate of 1.8 mL/min as carrier gas; for the MS detector ionization energy of 70 eV, a quadrupole temperature of 150°C and a source temperature of 230°C were applied. The injection port and mass transfer line temperatures were set at 250 and 280°C, respectively. The temperature program employed for this study was an initial temperature of 40°C held for 1.0 min, followed by an increase from 40 to 130°C at 15 grad/min and then from 130 to 300°C, where it was held for 6 min. 1.0 μL of samples/standards was injected in the pulsed splitless mode and data acquisition from the mass selective detector was done in the Scan mode. The *m/z* values for the ions selected for the quantitation were 141, 169, 120 and 184 for 4-chloro-*o*-toluidine, 4-aminobiphenyl, 2,4,5-trimethylaniline and benzidine, respectively.

Azo group reductive cleavage process. The sample materials were cut into very small pieces, from which 1.0 g was weighed into a reaction vessel with a fitting cover. Sampling of materials was done to give a true representation of parts that are in direct contact with the skin. Samples such as pants which have different dyed materials for the pockets were cut and mixed to homogeneity with the main material. 17 mL of 60 mM citrate buffer (pH 6.0) was added and heated at 70°C with continuous stirring. After 30 min, 3.0 mL of freshly prepared sodium dithionate was added, and the resulting solution heated for another 30 min at 70°C. The reaction vessel was then allowed to cool to room temperature before vacuum filtration, so as to prevent evaporative losses of low boiling analytes.

Extraction. The resulting solution from the reductive cleavage was filtered through a Buchner funnel into a vacuum flask and the fiber residue retained in the funnel was washed with four 10 mL aliquots of *tert*-butyl methyl ether. The collected filtrate was then transferred into a 100 mL separatory funnel and the vacuum flask was rinsed with additional 10 mL of *tert*-butyl methyl ether. The separatory funnel was shaken vigorously for the extraction of analytes and the phases were allowed to settle. The aqueous phase was discarded through the bottom opening of the separatory flask and the organic phase was transferred into a round bottom flask through the top opening of the separatory flask. *tert*-Butyl methyl ether was totally evaporated using a rotary evaporator operated below 50°C in order to prevent analyte evaporation. Then, 3.0 mL of *tert*-butyl methyl ether was used to dissolve the analyte residue after evaporation, filtered through a 0.45 μm polytetrafluoroethylene syringe filter into glass vials and analyzed by GC–MS.

RESULTS AND DISCUSSIONS

Quantification of aromatic amines. A 10 mg/L mixed standard solution of the analytes was injected to the GC–MS system and the total ion chromatogram obtained was used to identify and characterize individual analytes according to their retention times. The identification of analytes and their retention times were done using their extracted ion chromatograms and mass spectra. The extracted ion chromatograms of 4-chloro-*o*-toluidine (*m/z* 141), 4-aminobiphenyl (*m/z* 169), 2,4,5-trimethylaniline (*m/z* 120) and benzidine (*m/z* 184) in Fig. 1 show retention times of 6.97, 8.98, 6.89, and 9.86 min, respectively.

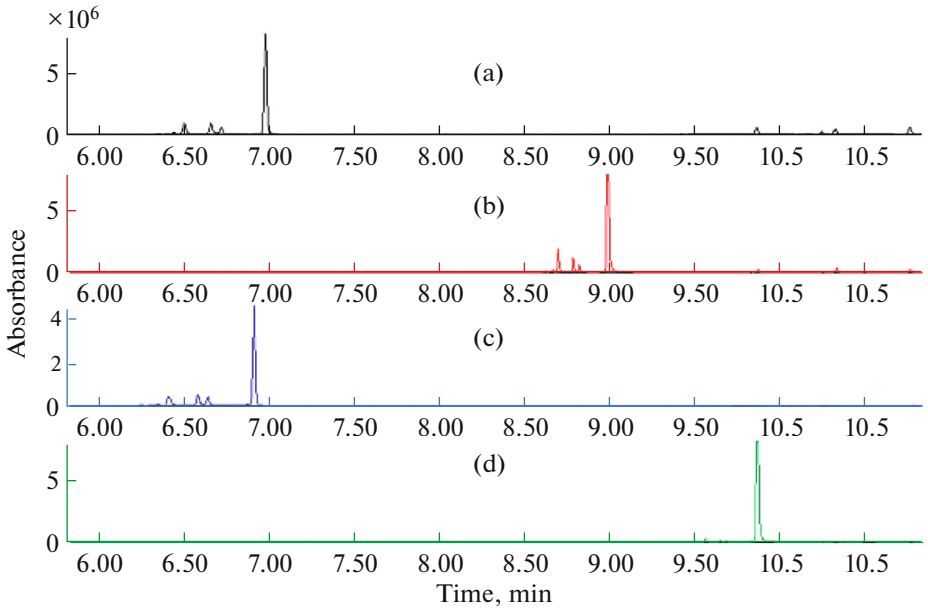

Fig. 1. Extracted ion chromatogram indicating retention times of aromatic amines: 4-chloro-*o*-toluidine (a), 4-aminobiphenyl (b), 2,4,5-trimethylaniline (c), benzidine (d).

Analytical figures of merit. The system performance of the GC–MS instrument was determined using standards in the concentration range of 2.0–50 mg/L. The GC–MS system performance for the analytes based on the limits of detection **(LOD)** and quantification **(LOQ)**, relative standard deviation **(RSD)** and correlation coefficient are presented in Table 1.

The LOD and LOQ were calculated according to Eqs. (1) and (2):

$$\mathrm{LOD} = 3\mathrm{SD}/m, \quad (1)$$

$$\mathrm{LOQ} = 10\mathrm{SD}/m, \quad (2)$$

where m is the slope and SD is the standard deviation of lowest concentration in the linear calibration plot.

The low RSD (in %) values obtained are indicative of high instrumental precision for all 4 analytes and the calibration plot showed good linearity according to the R^2 values. The limits of detection and quantification varied between 0.2–1.3 and 0.5–4 mg/L, respectively. These limits are satisfactory to perform compliance tests on azo dyed materials.

Recovery analysis. The purpose of performing the recovery test was to determine the percentage of the analytes that can be extracted from the citrate buffer and sodium dithionate mixture using the proposed extraction method which seeks to use a lesser volume of *tert*-butyl methyl ether than that stated in the standard methods. A sample solution was analyzed to ensure the analytes were not present or below the detection limit of the method. The aqueous sample solution was then spiked with the mixed standard of analytes. The spiking was done after the reductive cleavage action by sodium dithionate so as to maintain the integrity of the analytes in solution. The acetonitrile based mixed standard was ideal for the spiking process because its miscibility with water gave a true representation of dissolved amines in real sample solutions. Three replicate spiking experiments were performed to determine the percent recovery of each aromatic amine. The integrated peak areas of the analytes after extraction were used to calculate percent recoveries of 95 ± 2, 114 ± 4, 92 ± 3 and 100 ± 3 for 4-chloro-*o*-toluidine, 4-aminobiphenyl, 2,4,5-trimethylaniline and benzidine, respectively. The high recovery results

Table 1. Analytical figures of merit of GC–MS for selected aromatic amines

Analyte	LOD, mg/L	LOQ, mg/L	RSD, %	Linear range, mg/L	R^2
4-Chloro-*o*-toluidine	0.6	2.1	2.2	2.0–50	0.9993
4-Aminobiphenyl	0.9	2.9	3.4	2.0–20	0.9973
2,4,5-Trimethylaniline	1.3	4	3.4	2.0–50	0.9992
Benzidine	0.12	0.5	2.7	2.0–10	0.9997

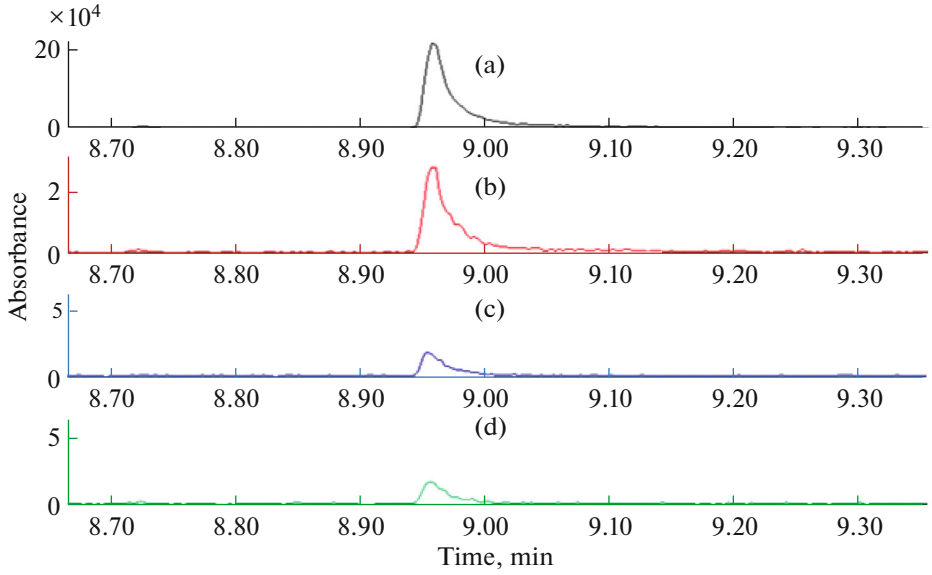

Fig. 2. Extracted ion chromatogram of *m/z* 169 (a), 170 (b), 115 (c) and 141 (d) indicating the occurrence of 4-aminobiphenyl in shoe sample.

show that the volume of *tert*-butyl methyl ether used is sufficient to extract analytes from the aqueous solution in a single extraction step. This also indicates that the proposed extraction method is suitable for the determination of aromatic amines in dyed products.

Real sample analysis. The method was applied to a variety of dyed products available on the market including gloves, pants, shoes, sandals and shirt samples. Particular attention was given to samples with dyed interior parts that are in contact with the skin when worn. The samples were made of such materials as cotton, wool, linen and silk. Samples with different interior and exterior compositions were also analyzed separately. None of the analytes under study were detected in all but one sample. 4-Aminobiphenyl, one of the four compounds listed to be directly carcinogenic to humans in the MAK III list was detected in a black shoe sample as shown by its extracted ion chromatogram in Fig. 2. To confirm this, three replicate extractions of the shoe sample after its reductive cleavage yielded similar results as shown in the overlay chromatograms of the major ion (*m/z* 169) in Fig. 3.

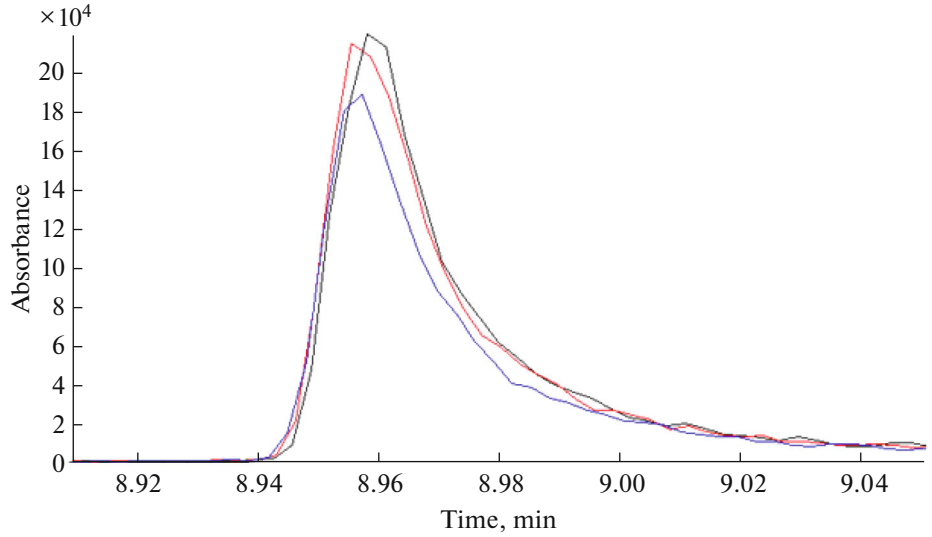

Fig. 3. Overlay chromatograms of *m/z* 169 for three replicate extractions of shoe sample.

The concentration of 4-aminobiphenyl in the shoe sample was well below 30 mg/kg limit and, therefore, did not cause alarm.

CONCLUSIONS

GC–MS is a reliable instrument for the separation, determination and quantification of the selected aromatic amines in this study. The system performance results obtained for the instrument were also satisfactory. The limits of detection and quantification were low enough for the limit set on hazardous aromatic amines, and the low RSD values also indicated high precision. The applicability of the extraction method to real samples was determined with a recovery test and the results obtained for all four analytes were close to a 100% recovery. The method was then applied to several dyed products on the market out of which 4-aminobiphenyl was detected in a shoe sample, but its concentration was below the limit set by regulatory bodies. It is believed that this method can be successfully extended for the simultaneous determination of the other harmful aromatic amines. The method presented in this study can be readily adopted by the least equipped and low budgeted laboratory for the determination of harmful aromatic amines.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest with this study.

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