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Assessment of water contamination caused by a mutagenic textile effluent/dyehouse effluent bearing disperse dyes

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ABSTRACT

High performance liquid chromatography coupled to a diode array detector method was developed to detect disperse dyes in water samples over the range 0.50–35 ng, with detection limits of 0.09 ng, 0.84 ng and 0.08 ng, respectively, with good repeatability and accuracy. This study identifies the disperse azo dyes C.I. Disperse Blue 373, C.I. Disperse Orange 37 and Disperse Violet 93 as components of a commercial dye formulation assigned as Dispersol Black Dye (CVS) used in the textile industry for dyeing synthetic fibers that are contributing to the mutagenicity found in the Cristais River, São Paulo, Brazil. High performance liquid chromatography coupled to a diode array detector was applied to monitor the occurrence of these dyes in: (1) the treated industrial effluent, (2) raw river water, (3) treated river water, and (4) the sludge produced by a Drinking Water Treatment Plant (DWTP) which is located 6 km downstream from the textile industrial discharge, where dyes' concentrations changed from 1.65 ng L^{-1} to 316 μ L^{-1}.

1. Introduction

Among the various industrial effluents, there is a growing concern regarding the potentially adverse effects of genotoxic textile dyes on aquatic biota and humans due to the contamination of water used for town supply and recreation. Wakabayashi and coworkers [1] demonstrated that some rivers in the world, especially in Europe, Asia and South America, are contaminated with potent frameshift-type and base substitution-type mutagens. These rivers were reported to be contaminated by either partially treated or untreated discharges from chemical industries, petrochemical industries, oil refineries, oil spills, rolling steel mills, untreated domestic sludge and pesticide runoff. But, although dyes are being released into the environment from multiple sources, there are very few studies in literature about their presence in bodies of water.

Synthetic dyes are extensively used in textile dyeing processes. The major classes of synthetic dyes are the azo type, which include reactive, disperse and acid dyes. Azo dyes correspond to 65% of the total production of dyes in the world [2,3]. Currently, the most used treatment for dye processing plant effluent is the biological treatment process, which is not efficient at removing dyes. Thus, dyes are often found chemically unchanged in the effluent of wastewater treatment plants; and these can both contaminate drinking water and/or become concentrated in the sludge causing a disposal problem.

Although the majority of commercial textile dyes are water-soluble, some dyes present hydrophobic behavior, especially the disperse dyes that are used for dyeing polyester fabrics [2,3]. Disperse dyes are non-ionic aromatic compounds bearing azo or anthraquinone as a chromophore group. The dyes are applied to the fibers by stable aqueous dispersion containing auxiliaries, especially dispersants at high temperature. Under this condition, the disperse dyes are dissolved, adsorbed onto the fiber surface and then transferred into the synthetic fiber. However, after the dyeing process finishes, the non-adsorbed dyes combine with the dispersing agents, which are components of the residual dyeing liquor and are sent to the treatment system. This process leads to an increased presence of dyes in wastewaters from textile processing plants including disperse dyes catalogued as being scarcely soluble in water. Some disperse dyes have also been shown to have a tendency to bioaccumulate.

Several types of dyes can have harmful effects on different organisms including humans, and some can be genotoxic in bacterial and mammalian assays [1]. Umbuzeiro et al. [4–6] reported that the effluent from a dye processing plant was causing the mutagenic activity detected in the waters of the Cristais River, São Paulo, Brazil. The authors also showed that a commercial dye

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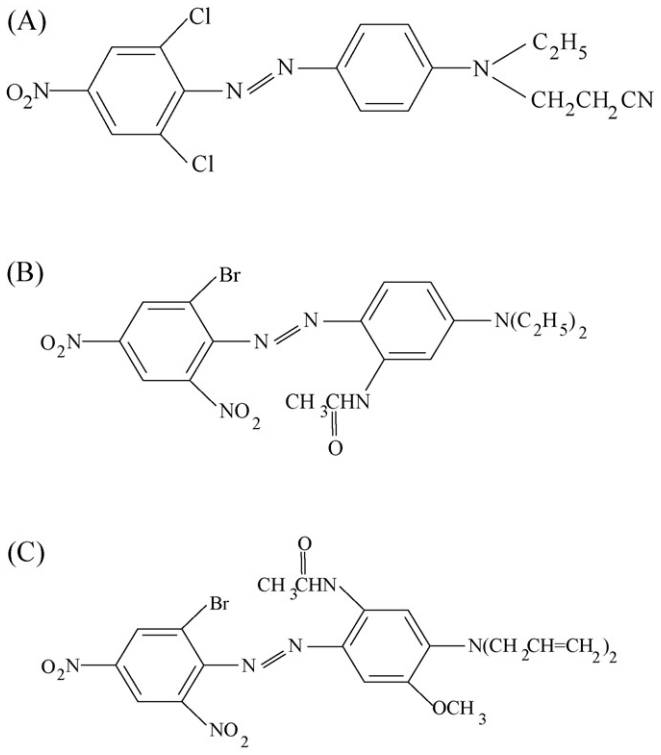

Fig. 1. Chemical structures of (A) C.I. Disperse Orange 37, (B) C.I. Disperse Violet 93 and (C) C.I. Disperse Blue 373.

designated in this work as Dispersol Black Dye (CVS) and widely used to dye polyester contributes to the detected mutagenicity. Previous work [4] has indicated that the commercial product CVS is composed of three disperse azo dyes: C.I. Disperse Blue 373 (DB373), C.I. Disperse Orange 37 (DO37) and Disperse Violet 93 (DV93), whose structures are shown in Fig. 1. Each dye (DB373, DO37 and DV93) presented mutagenic strengths of 6300, 4600, and 280 revertants/μg for YG1041 with S9, respectively [7]. The river water, sediment, drinking water and Drinking Water Treatment Plant sludge also presented mutagenic activity but the correlation of the mutagenic strength with the quantity of each of those dyes has not been done to date. In order to do that, it is necessary to have appropriate analytical techniques for detecting and quantifying low levels of these disperse azo dyes, especially in drinking water, which in this case serves a population of 60,000 inhabitants [8–10].

Several analytical methods have been proposed to detect the presence of some synthetic dyes in different matrices, mainly based on spectrophotometry [11–14], high performance liquid chromatography with different detectors [15–19] and mass spectrometry [20–26]. But cited works in the literature showing the possibility of disperse dyes monitoring in surface waters or wastewater from textile industry by HPLC diode array detectors, to our knowledge is rare [27].

The recognition of the health hazards of disperse azo dyes has highlighted the need to develop rapid and reliable analytical methods for evaluating it, with sufficient sensitivity to quantify it in water samples obtained from various relevant sampling sites. Therefore, the purpose of this work was to design and optimize an accurate and sensitive analytical method for monitoring dyes C.I. Disperse Blue 373, C.I. Disperse Orange 37 and C.I. Disperse Violet 93 using HPLC coupled with a diode array detector in environmental samples.

2. Experimental

2.1. Chemicals and isolation procedure of disperse-dye components in a commercial CVS sample

The CVS commercial dye sample was kindly provided by the textile dyeing industry. Stock solutions of the CVS dye were prepared by dissolving the commercial sample in acetonitrile (J.T. Baker) HPLC grade and deionized/demineralized water (Milli-Q® System – Millipore, Milford, MA, USA) in the proportion 50:50 (v/v). The dyes: Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 373 (DB373) (Fig. 1), chemical components of the commercial sample of CVS dye, were extracted in silica-gel 60H (Merck – 0.063–0.200 mm) compacted in a glass column. A mixture of hexane (Synth p.a.) and ethylacetate (Synth p.a.) (80:20, v/v) was used to elute the components of the CVS samples. The major fractions were purified in Sep-Pak C18 (Millipore, Milford, MA, USA) cartridges, pre-conditioned and eluted with acetonitrile. After the elution, the aliquots were dried in a rotating evaporator under a flow of ultrapure N_2, and then tested as to their purity using a high performance chromatography technique with diode array detection (HPLC-DAD-Shimadzu, model SCL-10AVP). Next each component was analyzed by mass spectrometry (UltrOTOFQ – ESI-TOF Mass Spectrometer – Bruker Daltonics, Billerica, MA, USA) and ^{1}H and ^{13}C NMR spectroscopy in DMSO-d_6 (Varian INOVA 500 spectrometers at 500 MHz). The chromatograms and obtained spectra confirmed the structures previously identified [4,6]. These samples of C.I. Disperse Orange 37 (DO37), C.I. Disperse Violet 93 (DV93) and C.I. Disperse Blue 373 (DB373) were used to construct analytical curves in order to spike the environmental samples and also to analyze the collected samples using the standard addition method.

2.2. Chromatographic analysis

The high performance liquid chromatographic analyses were carried out in a Shimadzu SCL-10AVP apparatus coupled with a diode array detector. The chromatograms were investigated between 200 and 800 nm, and the maximum wavelengths selected to analyze the disperse dyes DO37, DV93 and DB373 were 428, 562 and 592 nm, respectively. The HPLC analysis was performed in a reversed-phase column Shimadzu CLC-ODS (C18) (25 cm × 4.6 mm × 5 μm, 100 A) connected to a guard column Shimadzu CLC-ODS (C18) (1 cm × 4.6 mm × 5 μm, 100 A). All solutions were filtered before the analysis in a 0.45 μm PTFE filter. The best experimental conditions under optimized isocratic mode were: a mobile-phase acetonitrile/water 85:15 (v/v), a flow rate of 1.0 mL min^{-1} and a column temperature of 40 °C. The analysis time was 15 min, and all the analyses were carried out in triplicate. The results were compared with an HPLC coupled to a UV–vis detector (ProStar Varian HPLC apparatus with two high pressure gradient pumps (model 210/215) operating under isocratic conditions coupled to a UV–vis spectrophotometric detector (Varian ProStar 320).

2.3. Quantitative analysis

Taking into account the injected volumes, we calculated the respective masses of the dyes DO37, DV93 and DB373 and the analytical curves were obtained by plotting the peak area vs. amount (mass). The concentration of each disperse dye in the environmental sample was obtained by a linear regression of the analytical curve and confirmed by the standard addition method for each isolated dye. All the chromatographic procedures were carried out in triplicate for each analysis.

2.4. Analysis of environmental samples

We collected sediment, untreated and treated water from the Cristais River downstream from the effluent discharge of the textile dyeing facility. Raw and treated effluent from the textile dyeing facility and sludge from the Drinking Water Treatment Plant were also collected in March of 2008 [7]. For comparison, water and sediment samples were collected from a clean tributary of the Cristais River, which was upstream from the industrial discharge. Even though preliminary studies had identified the presence of the disperse dyes downstream from the effluent discharge of the dyeing industry, no quantification of the dyes had been performed [7]. In this work, the samples analyzed were described as follows: raw effluent from the dyeing industry (REDI); treated effluent from the dyeing industry (TEDI); raw river water (RW), which is taken in for treatment by the Drinking Water Treatment Plant located 6 km downstream from the textile industry discharge; pre-chlorinated water (PCW); drinking water (DW); sludge (SG) generated by the DWTP and sediment samples (SD) collected from where the water is taken in for treatment in the DWTP.

The collection of water and sediment for the chromatographic analysis was accomplished according to APHA [28] in properly washed glass flasks [29]. The samples were protected from light during the transportation and were stored under refrigeration. 100 L of the water samples were collected, concentrated and extracted in XAD4 resin (Sigma) as described elsewhere [7]. The extraction of 1 L of the industrial effluents (raw and treated) was performed using the liquid–liquid method [7]. The sediment and sludge samples were dried at 45 °C in the dark and 30 g of each sample were extracted using the ultra-sonication method [7]. All the extracts were reduced to small volumes using a rotary evaporator and were completely dried under ultra-pure nitrogen atmosphere and then dissolved in 100 μL of acetonitrile for chromatographic analysis.

3. Results and discussion

3.1. HPLC separation of CVS commercial dye sample

In order to optimize the best chromatographic elution, 20 μL of a mixture of standards of the commercial CVS dye sample at a concentration of 1.00 g L^{-1} was submitted to chromatographic separation at different electrolyte compositions. The chromatographic performance for dye separation in a mixture of acetonitrile + water at ratios of 80:15 (v/v); 80:20 (v/v) and 75:25 (v/v) were compared. Using some chromatographic parameters such as retention time (t_r), retention constant factor (k), selectivity (σ) and resolution between peaks (r), it was concluded that the best condition [30] for the separation of the investigated compounds was obtained at 85:15 (v/v) acetonitrile/water (ACN/H_2O), but other parameters were investigated to improve symmetry and to decrease the analysis time.

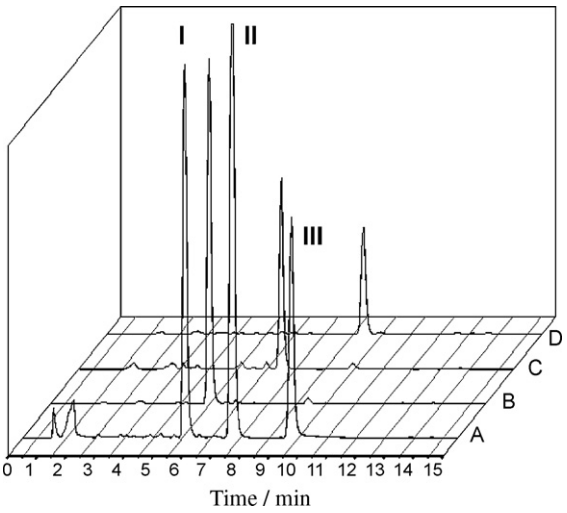

Fig. 2. Chromatographic profile of (A) commercial CVS sample, (B) Disperse Orange dye (DO37) obtained at 428 nm (peak I), (C) Disperse Violet dye (DV93) obtained at 562 nm (peak II), and (D) Disperse Blue dye (DB373) obtained at 592 nm (peak III). Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL min^{-1}.

The values of the peak area corresponding to each dye as a function of flow rate were analyzed at 0.8, 0.9, 1.0 and 1.2 mL min^{-1}. By evaluating several parameters such as: retention time (t_r), retention constant (k), peak resolution and theoretical plate number (N), a flow rate of 1.0 mL min^{-1} was chosen, as this allowed the column to perform well for the separation of these species and also gave good resolution for each peak.

The effect of column temperature was investigated at 25 °C, 30 °C, 35 °C and 40 °C; and by evaluating several parameters such as: retention time (t_r), retention constant (k), peak resolution and theoretical plate number, a temperature of 40 °C was chosen. This temperature gave good resolution, good symmetry and a short analysis time.

Thus, using these best experimental conditions, a typical chromatographic separation was obtained for the commercial CVS sample and the respective standard disperse dyes DO37, DV93 and DB373 by HPLC-DAD, as shown in Fig. 2. As can be seen, good

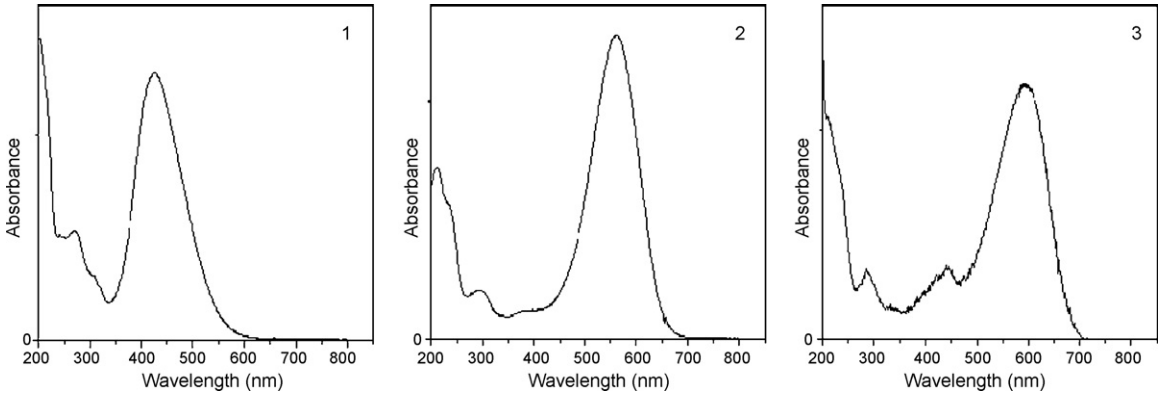

Fig. 3. UV–vis spectra obtained by diode array detection (DAD) under hydrodynamic condition (HPLC) of (1) Disperse Orange dye (DO37) obtained at 5.7 min, (2) Disperse Violet dye (DV93) obtained at 7.3 min and (3) Disperse Blue dye (DB373) obtained at 9.3 min. HPLC/DAD conditions: acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate of 1.0 mL min^{-1}.

chromatographic separations of the CVS sample (curve A) were obtained and each disperse dye (curves B, C and D) was monitored satisfactorily under the optimized conditions, which were ACN/H_2O 85:15% (v/v), a flow rate of 1 mL min^{-1} and a temperature of 40 °C. The elution of the examined compounds was completed in a chromatographic run of less than 15 min. Peak identification was based on the retention time (t_{r1} = 5.99 ± 0.17 min (DO37 – peak I), t_{r2} = 7.77 ± 0.55 min (DV93 – peak II) and t_{r3} = 9.70 ± 0.57 min (DB373 – peak III), which was confirmed by spiking authentic standard solutions of disperse dyes extracted from the commercial CVS sample as assigned in the experimental section. Characteristic UV–vis spectra obtained by the diode array detection under the hydrodynamic conditions were recorded and used as a parameter to identify and confirm the investigated species; and then afterwards to compare this with the one recorded for the pure samples of each component of the CVS sample. Comparing the chromatographic separations of the CVS sample, it is possible to conclude that the original commercial product is a mixture of three disperse dyes assigned as DO37, DV93 and DB373, confirming the separation observed previously by thin layer chromatography [4]. The spectra of the major components present in the CVS sample are displayed in Fig. 3, and the maximum absorbance found was 433 nm, 562 nm and 595 nm, for DO37, DV93 and DB373 dyes respectively; and these values were attributed to the azo group present in each molecule of dye. Taking into account the fact that each substance presents a different absorption pattern, the diode array detection confirmed the compounds by its UV–vis spectra. After confirmation, we proceeded with the quantitative analysis of the disperse dyes present in the commercial CVS sample.

3.2. Analytical evaluation

In quantitative spectrochemical analysis, according to IUPAC, the quantitative measure, *x*, of some spectral aspects of the analyte, such as spectral bands, can be seen. The concentration *c* or an amount *q* of the substance contained in the sample can be derived from the observed measurement. In general, the relationship of the measurement *x* as a function of the concentration *c* or of the amount *q* is called the analytical function. The plot of this analytical function is called the analytical curve.

In this context, analytical curves for the disperse dyes: Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 373 (DB373) were constructed by plotting the peak area *vs.* amount *m* (mass) of the dyes. These analytical curves were constructed for each dye with respect to their respective retention time and maximum wavelength. The isolated dyes were dissolved in acetonitrile to generate the standard stock solution and afterward these solutions were diluted to intermediate concentrations. Using an auto-injector, different volumes of these solutions were injected into the HPLC unit and the respective chromatogram was registered. The mass present in the volume injected was calculated each time according to the initial concentration. The analytical curves constructed from 1 to 20 μg of Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 373 (DB373), are shown in Fig. 4. An excellent linear relationship was obtained for all the disperse dyes in the region of 0.50–35 ng, which is shown in Table 1. The limit of detection (LOD) evaluated as the signal-to-noise ratio equal to 3:1 reaches values of around 0.09 ng (DO37); 0.84 ng (DV93) and 0.08 ng (DB373). The limit of quantification (LOQ) determined as the signal-to-noise ratio equal to 10:1 was calculated (LOQ = 10 × (SD/*B*)) and the values are around 0.27 ng (DO37 and DB373) and 0.84 ng (DV93). All the results are shown in Table 1. The repeatability of the proposed method, evaluated in terms of relative standard deviation, was measured as 3.2% (DO37), 4.1% (DV93) and 2.91% (DB373) over 10 experiments measuring samples containing 5 ng of each dye.

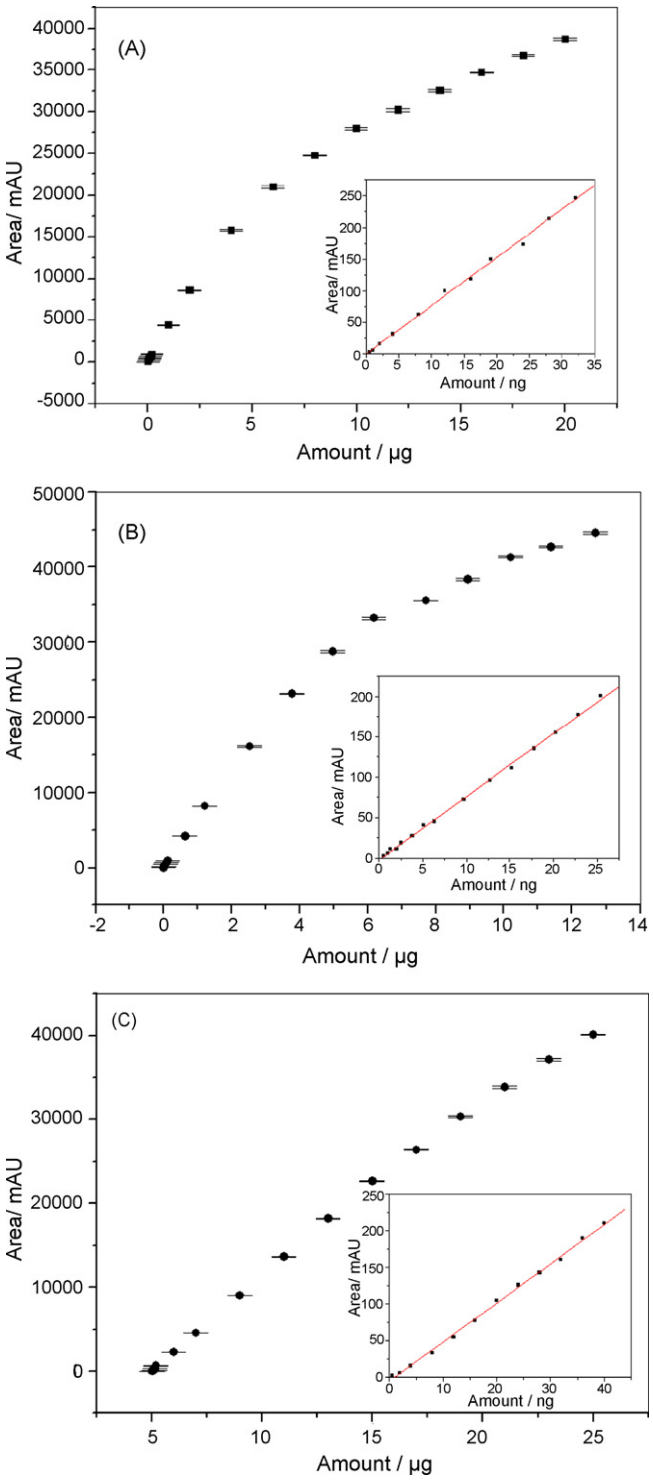

Fig. 4. Analytical curves obtained from disperse dyes present in the commercial CVS samples. (A) Disperse Orange dye (DO37) obtained at 5.7 min (428 nm), (B) Disperse Violet dye (DV93) obtained at 7.3 min (562 nm) and (C) Disperse Blue dye (DB373) obtained at 9.3 min (592 nm). HPLC/DAD conditions: acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate of 1.0 mL min^{-1}.

In order to test the method, a water sample from the tributary of the river containing no traces of dyes was spiked with 0.5–10 ng of each dye, with the aim of simulating the dye concentration levels that could be detected by the proposed method. Recoveries of 95% and 102% of DO37, DV93 and DB373 (VI) were obtained from the water samples (n = 3) using the proposed method. This is evi-

Table 1
Results obtained from the analytical curves for the chromatographic determination of disperse dyes present in the commercial CVS dye using the HPLC-DAD technique in the range of 0.5–35 ng; and limits of detection and quantification obtained for the proposed methodology.

Dye	Linearity	r^2	LOD (ng)	LOQ (ng)
DO37	$A = 505.25 + 6057.64m$	0.9995	0.089	0.268
DV93	$A = 829.62 + 4972.30m$	0.9992	0.835	0.835
DB373	$A = -765.75 + 6252.64m$	0.9998	0.089	0.260

A: area (mAU), m: amount (ng), r^2: correlation coefficient, LOD: limit of detection, LOQ: limit of quantification.

dence of the accuracy of the proposed procedure. The statistical calculations for the assay results showed suitable precision of the HPLC-DAD method. According to the *t*-test, there were no significant differences between the calculated and added concentrations at the 95% confidence level, being within an acceptable range of error, indicating that the proposed method could be used for detection and determination of CVS in wastewater samples. Therefore, the proposed method was employed for determining these disperse dyes in the environmental samples.

3.3. Detection and quantification of CVS dye components in environmental samples

The developed method was applied to determine the disperse-dye content in the environmental sample extracts. The same extracts have shown mutagenic activity in *Salmonella*/microsome assays as previously published [6–9]. Aliquots of 20 μL were injected directly into the HPLC system coupled with diode array detection.

Fig. 5 shows a typical chromatographic determination obtained for the disperse dyes in the environmental samples. The presence of these disperse dyes can be detected in all samples analyzed except for the raw water and sediment collected upstream from the dyeing-effluent discharge. These do not show any chromatographic signal attributed to presence of dye at retention time (negative control). All the results were confirmed by the standard addition of Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 373 (DB373), using the averages of the values obtained in the triplicate experiments. The quantitative results obtained for the environmental samples tested are shown in Tables 2–4. It is possible to observe that the treatment used by the dyeing textile facility was not efficient in removing the components of the CVS dyes (Table 2). Because the three dyes are mutagenic, they

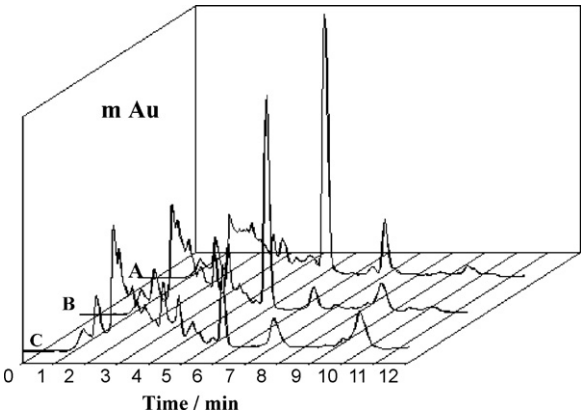

Fig. 5. Chromatographic profile of (A) river water used by the DWTP; (B) pre-chlorinated water, (C) drinking water, where it is detected (I) Disperse Orange dye (DO37); (II) Disperse Violet dye (DV93) and (III) Disperse Blue dye (DB373). Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL.

Table 2
Determination of the CVS-dye components in effluents samples collected from textile industry by CLAE-DAD. Disperse Orange dye (DO37) identified at 5.7 min (428 nm), disperse Violet dye (DV93) identified at 7.3 min (562 nm) and disperse Blue dye (DB373) identified at 9.3 min (592 nm). HPLC/DAD conditions: Acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate of 1.0 mL min^{-1}.

Samples	DO37 (μg L^{-1})	DV93 (μg L^{-1})	DB373 (μg L^{-1})
REDI	316 ± 3.08	12.0 ± 2.09	57.9 ± 1.27
TEDI	126 ± 2.12	6.03 ± 2.58	67.0 ± 1.13

REDI: raw effluent from dyeing industry; TEDI: treated effluent from dyeing industry.

Table 3
Determination of the CVS-dye components in sediments and sludge samples by CLAE-DAD. Disperse Orange dye (DO37) identified at 5.7 min (428 nm), disperse Violet dye (DV93) identified at 7.3 min (562 nm) and disperse Blue dye (DB373) identified at 9.3 min (592 nm). HPLC/DAD conditions: acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate of 1.0 mL min^{-1}.

Samples	DO37 (ng g^{-1})	DV93 (ng g^{-1})	DB373 (ng g^{-1})
SD	27.4 ± 1.07	3.38 ± 0.74	41.0 ± 0.20
Samples	DO37 (μg g^{-1})	DV93 (μg g^{-1})	DB373 (μg g^{-1})
SG	5.44 ± 0.18	0.12 ± 0.02	1.85 ± 0.71

SD: river sediment collected where the water is taken in by the Drinking Water Treatment Plant (DWTP); SG: sludge generated in the DWTP.

Table 4
Determination of the CVS-dye components in surface water samples by CLAE-DAD. Disperse Orange dye (DO37) identified at 5.7 min (428 nm), disperse Violet dye (DV93) identified at 7.3 min (562 nm) and disperse Blue dye (DB373) identified at 9.3 min (592 nm). HPLC/DAD conditions: acetonitrile/water 85:15 as mobile-phase, C18 column as stationary-phase, oven temperature 40 °C, flow rate of 1.0 mL min^{-1}.

Samples	DO37 (ng L^{-1})	DV93 (ng L^{-1})	DB373 (ng L^{-1})
RW	397 ± 9.14	11.8 ± 0.66	64.9 ± 1.97
SPCW	43.5 ± 3.29	2.93 ± 0.08	43.2 ± 0.40
STW	8.86 ± 0.26	3.05 ± 0.36	1.65 ± 0.16

RW: river water used by the DWTP; PCW: pre-chlorinated water; DW: drinking water.

account for at least part of the mutagenic activity detected in the same samples [9]. The three dyes had been transported along the river since they were detected in the river water (Table 4) and sediment sample (Table 3). Even after the pre-chlorination step and final treatment, they were still detected, although in lower concentrations (Table 4). The treatment applied by the DWTP [9] was able to partially remove the dyes as can be observed for the DWTP sludge sample (Table 3), but small amounts were detected in the final drinking water where textile dyeing effluents are discharged.

Increased awareness of the harmful effects of these dyes in surface waters has also resulted due to the chemical structure properties of these dyes, that can be considered collectively as adsorbable organic halides (AOX) [31]. The toxic effects of AOX range from carcinogenicity and mutagenicity to acute and chronic toxicity and the discharge limit has been submitted to stringent regulations.

4. Conclusions

Our findings show that HPLC coupled to a diode array detector can be an excellent alternative to determine Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 37 in water samples over the range 0.50–35 ng, with detection limits of 0.09 ng, 0.84 ng and 0.08 ng, respectively, with good repeatability and accuracy. The proposed method can be used to monitor the occurrence of three disperse dyes bearing nitro, azo and amine groups as sub-

stituent in: (1) the treated industrial effluent, (2) raw river water, (3) treated river water, and (4) the sludge produced by a Drinking Water Treatment Plant (DWTP), where concentrations changed from 1.65 ng L^{-1} to 316 μg L^{-1}. The work concludes that Disperse Orange 37 (DO37), Disperse Violet 93 (DV93) and Disperse Blue 373 (DB373) are present in untreated river water and also in drinking water, indicating that the adopted effluent treatment based on pre-chlorination, flocculation, coagulation and flotation, commonly used by DWTP, is not completely efficient to remove these dyes, which can be corroborated with mutagenic activity detected in this wastewaters.

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References

[1] T. Ohe, T. Watanabe, K. Wakabayashi, Mutagens in surface waters: a review, Mutat. Res. 567 (2004) 109–149.
[2] H. Zollinger, Color Chemistry – Synthesis, Properties and Applications of Organic Dyes and Pigments, V.C.H. Publishers, New York, 1991.
[3] C.C.I. Guaratini, M.V.B. Zanoni, Corantes têxteis, Quím. Nova 23 (2000) 71–78.
[4] G.A. Umbuzeiro, H. Freeman, S. Warren, D.P. Oliveira, M. Sakagami, T. Watanabe, L.D. Claxton, Contribution of azo dyes to the mutagenic activity detected in the Cristais rivers waters, Chemosphere 60 (2005) 55–64.
[5] G.A. Umbuzeiro, D. Roubicek, C.M. Rech, M.I. Sato, L.D. Claxton, Investigating the sources of the mutagenic activity found in a river using the Salmonella assay and different water extraction procedure, Chemosphere 54 (2003) 1589–1597.
[6] D.P. Oliveira, P.A. Carneiro, C.M. Rech, M.V.B. Zanoni, L.D. Claxton, G.A. Umbuzeiro, Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water, Environ. Sci. Technol. 40 (2006) 6682–6689.
[7] D.P. Oliveira, P.A. Carneiro, M.M. Sakagami, M.V.B. Zanoni, G.A. Umbuzeiro, Chemical characterization of a dye processing plant effluent – identification of the mutagenic components, Mutat. Res. Gen. Toxicol. Environ. Mutag. 626 (2007) 135–142.
[8] D.P. Oliveira, M.L. Kuhlmann, G.A. Umbuzeiro, Evaluation of the presence of mutagenic dyes in sediments from Cristais river, J. Soil Sediment Contam. 15 (2006) 455–462.
[9] D.P. Oliveira, P.A. Carneiro, M.M. Sakagami, M.V.B. Zanoni, G.A. Umbuzeiro, Chemical characterization of a dye processing plant effluent – identification of the mutagenic components, Mutat. Res. 626 (2007) 135–142.
[10] R.O.A. Lima, A.P. Bazo, D.M.F. Salvadori, C.M. Rech, D.P. Oliveira, G.A. Umbuzeiro, Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source, Mutat. Res. 626 (2007) 53–60.
[11] P.M. Urquiza, J.L. Beltrán, Determination of the dissociation constants of sulfonated azo dyes by capillary zone electrophoresis and spectrophotometry methods, J. Chromatogr. A 917 (2001) 331–336.
[12] S. Sxahin, C. Demir, S. Guçer, Simultaneous UV–vis spectrophotometric determination of disperse dyes in textile wastewater by partial least squares and principal component regression, Dyes Pig. 73 (2007) 368–376.
[13] C.V. Anibal, M. Odena, I. Ruisanchez, M.P. Callao, Determinaiton the adulteration of species with sudan I, II, III and IV dyes by UV visible spectroscopy and multivariate classification techniques, Talanta 79 (2009) 887–892.
[14] M. Balakrishan, S. Arul Antony, S. Gunasekaran, R.K. Natarajan, Impact of dyeing industrial effluents on the groundwater quality in Kancheepuram, Indian J. Sci. Technol. 1 (2008) 1–8.
[15] R. Noguerol-Cal, J.M. López-Villarino, G. Fernandez-Martinez, L. Barral-Losada, M.V. Gonzalez-Rodriguez, High-performance liquid chromatography analysis of ten dyes for control of safety of commercial articles, J. Chromatogr. A 1179 (2008) 152–160.
[16] C.S. Eskilsson, R.L. Davidsson, L. Mathiasson, Harmful azo colorants in leather. Determination based on their cleavage and extraction of corresponding carcinogenic aromatic amines using modern extraction techniques, J. Chromatogr. A 955 (2002) 215–227.
[17] A. Pielesz, I. Baranowska, A. Rybak, A. Wlochowicz, Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes, Ecotoxicol. Environ. Saf. 53 (2002) 42–47.
[18] T.M. Mazzo, A.A. Saczk, G.A. Umbuzeiro, M.V.B. Zanoni, Analysis of aromatic amines in surface waters receiving wastewater from a textile industry by liquid chromatographic with electrochemical detection, Anal. Lett. 39 (2006) 2671–2685.
[19] S.J. Sppeers, B.H. Little, M. Roy, Separation of acid, basic and dispersed dyes by a single gradient elution reversed phase high performance liquid chromatography system, J. Chromatogr. A 674 (1994) 263–270.
[20] C. Baiocchi, M.C. Brussino, E. Pramauro, A.B. Prevot, M.G. Palmisano, Characterization of methyl orange and its photocatalytic degradation products by HPLC/UV–vis diode array and atmospheric pressure ionization quadrupole ion trap mass spectrometry, Int. J. Mass Spectrom. 214 (2002) 247–256.
[21] A. Plum, G. Braun, A. Rehorek, Process monitoring of anaerobic azo dyedegradation by high-performance liquid chromatography–diode array detection continuously coupled to membrane filtration sampling modules, J. Chromatogr. A 987 (2003) 395–402.
[22] A. Rehorek, K. Urbig, R. Meurer, C. Schafer, A. Plumg, Braun, monitoring of azo dye degradation processes in a bioreactor by on-line high-performance liquid chromatography, J. Chromatogr. A 949 (2002) 263–268.
[23] M.R. Fuh, K.J. Chia, Determination of sulphonated azo dyes in food by ion pair liquid chromatography with photodiode array and electrospray mass spectrometry detection, Talanta 56 (2002) 663–671.
[24] G.A. Lord, D.B. Ordon, L.W. Tetler, C.M. Carr, Electrochromatography-electrospray mass spectrometry of textile dyes, J. Chromatogr. A 700 (1995) 27–33.
[25] F. Calbiani, M. Careri, L. Elviri, A. Mangia, I. Zagnoni, Accurate massmeasurements for the confirmation of sudan azo-dyes in hot chilli products by capillary liquidchromatography–electrospray tandem quadrupole orthogonal-acceleration time of flight mass spectrometry, J. Chromatogr. A 1058 (2004) 127–135.
[26] M. Holcïapek, P. Jandera, J. Prikryl, Analysis of sulphonated dyes and intermediates by electrospray mass spectrometry, Dyes Pigments 43 (1999) 127–137.
[27] A. Reife, H.S. Freeman, Environmental Chemistry of Dyes and Pigments, Wiley Interscience, New York, 1995.
[28] APHA-Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.
[29] US Environmental Protection Agency, Office of Water. Manual for the Certification of Laboratories Analyzing Drinking Water. Criteria and Procedures, 45268. EPA 815 B-97-001, March 1997, Cincinnati, USA.
[30] L.R. Snyder, J.J. Kirkland, J.L. Glajch, Pratical HPLC Method Development, 2nd ed., John Wiley, New York, 1997.
[31] N.S. Deshmukh, K.L. Lapsiya, D.V. Savant, S.A. Chiplonkar, T.y. Teole, P.K. Dhakephalkar, D.R. Ranade, Upflow anaerobic filter for the degradation of adsorbable organic halides (AOX) from bleach composite wastewater of pulp and paper industry, Chemosphere 75 (2009) 1179–1185.