Using SPE-LC-ESI-MS/MS Analysis to Assess Disperse Dyes in Environmental Water Samples

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We have optimized an SPE-LC-ESI-MS/MS method and used it to monitor disperse dyes in environmental aquatic samples. Calibration curves constructed for nine disperse dyes—Red 1, Violet 93, Blue 373, Orange 1, Orange 3, Orange 25, Yellow 3, Yellow 7 and Red 13—in aqueous solution presented good linearity between 2.0 and 100.0 ng mL⁻¹. The method provided limits of detection and quantification around 2.0 and 8.0 ng L⁻¹, respectively. For dyes at concentrations of 25.0 ng mL⁻¹, the intra- and interday analyses afforded relative standard deviation lower than 6 and 13%, respectively. The recovery values obtained for each target analyte in Milli-Q water, receiving waters and treated water samples spiked with the nine studied dyes at concentrations of 8.0, 25.0 and 50.0 ng L⁻¹ (n = 3) gave average recoveries greater than 70%, with RSD <20%. Statistical evaluation aided method validation. The validated method proved to be useful for analysis of organic extracts from effluents and receiving water samples after an SPE extraction step. More specifically, the method enabled detection of the dyes Disperse Red 1, Disperse Blue 373 and Disperse Violet 93 at concentrations ranging from 84 to 3452 ng L⁻¹ in the treated effluent (TE), affluent and points collected upstream and downstream of the drinking water treatment plant of a textile dye industry in Brazil.

Introduction

The last few decades have seen growing global concern over the discharge of textile industrial wastewater containing a huge amount of dyes into the environment. Indeed, industrial water treatment fails to remove this type of contaminants completely, so the dyes may reach surface waters and drinking water treatment plants (1, 2). The presence of these compounds in surface waters limits the application of these waters in recreation and irrigation, renders the waters inadequate for human consumption and affects the aquatic biota negatively (3–5). In 2010, the International Agency of Research on Cancer (IARC) recognized that some non-ionic azo dyes have mutagenic and carcinogenic activity (6).

Ohe et al. (1) have reviewed the genotoxic and/or carcinogenic hazards of natural waters in several places worldwide. These authors found that the presence of dyes and derivatives correlated with a range of industrial and municipal sources, and they emphasized the need to identify which sources generated these contaminants. Some authors have demonstrated that a number of dyes are toxic to aquatic life (6–9). In addition, the partial degradation of azo dyes can produce free aromatic amines that are potentially carcinogenic and mutagenic (10, 11), and whose transformation products may harm aquatic organisms (12).

Disperse dyes are low-molecular-weight synthetic organic dyes that represent at least 22% of the dyes consumed in the world (1). They are sparingly soluble in water, and the textile industry usually employs them in colloidal dispersions, to obtain hydrophobic textile fibers such as acetate, acrylic, nylon, polyester and polyurethane fibers. Most disperse dyes are essentially planar compounds bearing an azo chromophore attached to polar functional groups such as amine, nitro and halogens like chlorine or bromine. Increased awareness of the negative environmental effects of azo dyes has demanded the design of several analytical methods to help detect and determine these contaminants. Today, analytical methods to monitor disperse dyes are scarce, a result of the particular physicochemical properties of these substances. The currently available methods rely on spectrophotometry (13, 14) and chromatography coupled to a diode array UV-Vis detector (15–17). Unfortunately, these methods cannot detect azo dyes at very low concentrations in surface waters, so analysis of this kind of sample usually requires large wastewater volume and a laborious pre-concentration step.

Despite the high detectability and sensitivity of liquid chromatography coupled to mass spectrometry, analysts have rarely applied this method to analyze disperse dyes. Literature works (18, 19) have reported on the use of solid-phase extraction (SPE)—high-performance liquid chromatography (HPLC) coupled with electrospray tandem mass spectrometry to determine nine sensitizing disperse dyes. In these works, the authors reached detection limits that ranged from 0.05 to 2.48 μg L⁻¹ and employed the developed method to monitor contaminants in dying wastewater. Petrick et al. (20) also used HPLC coupled to electrospray ionization mass spectrometry to identify 15 basic and 13 disperse dye standards extracted from acrylic fibers. Researchers have applied liquid chromatography/electrospray ionization mass spectrometry operating in the negative/positive ion switching mode and thermostap HPLC/mass spectrometry (21–25) to determine disperse dyes extracted from polyester and cellulose acetate fibers. To the best of our knowledge, there has been no research into methods based on LC–MS-MS to quantify disperse dyes at low levels in environmental aquatic samples such as river waters and wastewaters.

Considering the complex physicochemical characteristics of textile dyes and the potential toxicity and/or mutagenicity of disperse dyes bearing azo groups and disperse azo dyes derivatives, this work aimed to develop and optimize a method based on SPE.
and liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS/MS), to determine and quantify low concentrations of disperse azo dyes simultaneously and to identify disperse dyes residues in environmental water samples. We monitored nine disperse dyes: C.I. Disperse Red 1, C.I. Disperse Violet 93, C.I. Disperse Blue 373, C.I. Disperse Orange 1, C.I. Disperse Orange 3, C.I. Disperse Orange 25, C.I. Disperse Yellow 3, C.I. Disperse Yellow 7 and C.I. Disperse Red 13 (Figure 1). These dyes constitute disperse dye models with wide application in synthetic fibers and recognized potential hazards. Statistical analysis helped to validate the proposed method, which in turn aided analysis of organic extracts from effluents and receiving water samples after an SPE clean-up step. Analysis of these dyes at very low levels is relevant and offers subsidies for better understanding of their transport processes, persistence, bioaccumulation and biotransformation in the environment or during human metabolism.

Experimental

Chemicals

The standards of the dyes C.I. Disperse Red 1, C.I. Disperse Orange 1, C.I. Disperse Orange 3, C.I. Disperse Orange 25, C.I. Disperse Yellow 3, C.I. Disperse Yellow 7 and C.I. Disperse Red 13 were obtained from Sigma-Aldrich. The dyes C.I. Disperse Violet 93 and C.I. Disperse Blue 373 (chemical structures in Figure 1) were isolated and purified from a commercial dye sample as described previously (17). Acetonitrile (ACN), methanol (MeOH) and dichloromethane (DCM) were purchased from J. T. Baker. Milli-Q water (18 \( \mu \) S cm \(^{-1} \)) and formic acid 98% was acquired from J.T. Baker. Millipore Inc., USA. Formic acid 98% was acquired from J.T. Baker, USA.

Instrumentation and reagents

Standard solutions and calibration curves

Stock MeOH solutions of each dye were prepared at a concentration of 100 \( \mu \)g mL\(^{-1} \). For LC-ESI-MS/MS analysis, mixtures of the standards were prepared by appropriate dilutions at concentrations of 0.5, 1.0, 2.0, 5.0, 7.5, 10.0, 25.0, 50.0 and 100.0 ng mL\(^{-1} \). The analytical curves were constructed by plotting the peak area in counts versus the concentration in ng mL\(^{-1} \). (Phenomenex, USA) previously conditioned with Milli-Q water and MeOH. The pH of the samples was adjusted to \( \sim 3.5 \) with the aid of 2% formic acid. Each sample was divided into eight replicate aliquots (250 mL). To evaluate SPE recoveries, the replicate aliquots were spiked with a mixture of analyte standards at concentrations ranging from 8.0, 25.0 and 50.0 ng L\(^{-1} \) before extraction. The SPE cartridges, which were positioned on a vacuum manifold, were preconditioned with 6 mL of MeOH and 6 mL of Milli-Q water at a flow rate of 1 mL min\(^{-1} \). Then, the samples were eluted through the SPE cartridges at a flow rate around 1 mL min\(^{-1} \). Next, the cartridges were washed with 10 mL of Milli-Q water and 5 mL of MeOH/Milli-Q water (20:80, v/v). The retained dyes were eluted with 5 mL of ACN/MeOH (1:2) and 5 mL of DCM/MeOH 1:1, at a flow rate of 10 mL min\(^{-1} \). The extracts were evaporated to dryness under a gentle nitrogen stream. Before LC-ESI-MS/MS analysis, all the samples were reconstituted with 1.0 mL of MeOH/water (9:1, v/v).

LC-ESI-MS/MS analysis

LC-ESI-MS/MS analyses were conducted on an Agilent 1200 Series HPLC system coupled with a hybrid quadrupole linear ion trap mass spectrometer (3200 Q Trap, Applied Biosystems/ MDS Sciex) and equipped with a ZORBAX Eclipse XDB-C18 column (4.6 \( \times \) 150 mm; 5 \( \mu \)m).

The disperse dyes were analyzed by LC-ESI-MS/MS. The following solvent gradient program was used: initial water/MeOH 1:1 solvent composition (both solvents contained formic acid 0.1%), programed to increase the amount of MeOH to 100% linearly within 16 min. To clean the column, the MeOH concentration was kept constant at 100% for 9 min; the re-equilibration time was 5.0 min. The mobile phase flow rate was 500 \( \mu \)L min\(^{-1} \); the injection volume was 40 \( \mu \)L. The temperature of the ESI heater was set to 500°C. The dyes were analyzed in the selected reaction monitoring (SRM) mode with electrospray ionization in the positive mode. The MS/MS parameters such as declustering potential (DP) and collision energy (CE) were optimized for each compound by direct injection. The optimized ion source parameters were evaluated at the following settings: curtain gas (CUR) = 18 psi, ion spray voltage (IS) = 5000 V, nebulizer gas (GS1) = 40 psi, turboheater gas (GS2) = 50 psi and temperature (TEM) = 500°C. The collision-activated dissociation (CAD) gas was set to high pressure. All the samples were injected in triplicate. The analytes found in the environmental samples were quantified by extrapolation from the calibration curve. The positive samples were confirmed by calculating the peak area ratios between the quantification (Q) and confirmation (q1 and q2) transitions and comparing them with ion ratios obtained from a reference standard. The sample was considered positive when the experimental ion ratio fell within the tolerance range, according to the EU Decision 2002/657/EC (26).

Results

Disperse dyes standards: analytical parameters optimization

A mixture of the standards of the selected disperse dyes—C.I. Disperse Red 1, C.I. Disperse Violet 93, C.I. Disperse Blue 373, C.I. Disperse Orange 1, C.I. Disperse Orange 3, C.I. Disperse Orange 25, C.I. Disperse Yellow 3, C.I. Disperse Yellow 7 and...
C.I. Disperse Red 13—in MeOH/H2O 50:50 (v/v) at a concentration of 200 ng mL\(^{-1}\) each helped to optimize the SRM transitions and MS parameters for the LC-ESI-MS/MS analysis of the dyes by direct injection (ESI-MS/MS). Table I lists the best instrumental conditions for the unequivocal identification of the target textile dyes; the results correlate with the structure of the analytes. The spectra of all the compounds revealed an abundant [M + H]\(^+\) ion, selected as precursor ion. More specifically, we monitored three product ions (transitions) for each dye (Table I). As for Disperse Yellow 7 and Disperse Orange 25, they exhibited poor fragmentation for the third transition, so it was only possible to monitor the two main transitions of these dyes.

Fragmentation of the ions produced the expected product ions with higher abundance. This enabled acquisition of other sensitive transitions (see Table I. Q—quantification transitions, q1 and q2—qualification transition for each dye). We avoided non-specific fragmentations as much as possible, to minimize the risk of false positives (27). For example, the dye Disperse Orange 3 exhibited the third transition at \(m/z\) 243.1 > 197.1, which corresponded to a neutral loss of \(m/z\) 46.0 Da, due to NO\(_2\). Because NO\(_2\) exists in seven of the nine dyes studied here, it constitutes a non-specific fragmentation; hence, it was necessary to avoid it. To confirm an attribution, the EU guidelines (26) advocate acquisition of at least two specific transitions for each compound; in other words, two is the minimum number of identification points (IPs) required for a safe confirmation.

To confirm the target dyes and avoid false positive results during quantitative determinations, this study followed the EU guidelines for LC–MS-MS analysis (Commission Decision 2002/657/EC) (26): apart from the acquisition of two SRM transitions for each compound, we had to consider the retention time and to monitor the SRM ratio (which is the relationship between the abundance of the area of the transitions selected for quantification and identification, Q/q). Table I contains the values of the optimized parameters and of the SRM transitions used to confirm and quantify all the target dyes. Table I also presents the relative abundances of the SRM transitions monitored for each compound. The values of two SRM ratios obtained for two selected analytes in the sample gave a coefficient of variation (relative standard deviation, RSD) around ±20%, indicating reliable values.

To corroborate the identity of each dye, we calculated the relationship between the peak area ratios attained from

### Table I

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Retention time [min]</th>
<th>[M + H](^+)</th>
<th>Q—Quantification transition</th>
<th>DP (V)</th>
<th>CE (eV)</th>
<th>q1 confirmation transition</th>
<th>DP (V)</th>
<th>CE (eV)</th>
<th>q2 confirmation transition</th>
<th>DP (V)</th>
<th>CE (eV)</th>
<th>Ion ratio 1</th>
<th>Ion ratio 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disperse Orange 3</td>
<td>13.8</td>
<td>243.1</td>
<td>243.1 &gt; 121.9</td>
<td>36</td>
<td>23</td>
<td>243.1 &gt; 135.0</td>
<td>36</td>
<td>15</td>
<td>270.1 &gt; 150.2</td>
<td>41</td>
<td>21</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Disperse Yellow 3</td>
<td>16.1</td>
<td>270.1</td>
<td>270.1 &gt; 107.0</td>
<td>41</td>
<td>29</td>
<td>270.1 &gt; 108.1</td>
<td>41</td>
<td>41</td>
<td>270.1 &gt; 150.2</td>
<td>41</td>
<td>21</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Disperse Orange 25</td>
<td>16.4</td>
<td>324.1</td>
<td>324.1 &gt; 282.9</td>
<td>36</td>
<td>25</td>
<td>324.1 &gt; 148.9</td>
<td>36</td>
<td>25</td>
<td>270.1 &gt; 150.2</td>
<td>41</td>
<td>21</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>Disperse Red 1</td>
<td>16.5</td>
<td>315.0</td>
<td>315.0 &gt; 134.3</td>
<td>61</td>
<td>31</td>
<td>315.0 &gt; 108.0</td>
<td>61</td>
<td>57</td>
<td>315.0 &gt; 255.2</td>
<td>61</td>
<td>39</td>
<td>1.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Disperse Red 13</td>
<td>18.3</td>
<td>350.0</td>
<td>350.0 &gt; 134.1</td>
<td>56</td>
<td>37</td>
<td>350.0 &gt; 290.0</td>
<td>56</td>
<td>29</td>
<td>350.0 &gt; 134.9</td>
<td>56</td>
<td>33</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Disperse Orange 1</td>
<td>19.4</td>
<td>319.0</td>
<td>319.0 &gt; 269.1</td>
<td>61</td>
<td>27</td>
<td>319.0 &gt; 222.2</td>
<td>61</td>
<td>29</td>
<td>319.0 &gt; 168.1</td>
<td>61</td>
<td>49</td>
<td>1.6</td>
<td>5.7</td>
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<tr>
<td>Disperse Violet 93</td>
<td>20.0</td>
<td>480.1</td>
<td>480.1 &gt; 192.2</td>
<td>56</td>
<td>39</td>
<td>480.1 &gt; 191.0</td>
<td>56</td>
<td>39</td>
<td>480.1 &gt; 207.2</td>
<td>56</td>
<td>25</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Disperse Yellow 7</td>
<td>20.7</td>
<td>317.0</td>
<td>317.0 &gt; 105.1</td>
<td>51</td>
<td>27</td>
<td>317.0 &gt; 181.2</td>
<td>51</td>
<td>21</td>
<td>533.9 &gt; 245.9</td>
<td>61</td>
<td>37</td>
<td>2.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Disperse Blue 373</td>
<td>21.2</td>
<td>533.9</td>
<td>533.9 &gt; 261.2</td>
<td>61</td>
<td>25</td>
<td>533.9 &gt; 260.2</td>
<td>61</td>
<td>25</td>
<td>533.9 &gt; 255.2</td>
<td>61</td>
<td>37</td>
<td>2.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Q, Quantification transition; q, confirmation transition; DP, declustering potential; CE, collision energy.

Figure 1. Chemical structure of the disperse azo dyes analyzed in this study.
quantification transitions (Q) and confirmation transitions (q1 and q2) for each compound and compared them with the ion ratios obtained from a reference standard including the relationship between Q and q (Table I). This confirmed positive findings on the basis of the relationship between Q/q and the ion ratios. The dye Disperse Red 1 will help to illustrate this procedure—this dye presented Q—m/z 315.0 > 134.3 (DP = 61.0 V, CE = 31.0 eV) with respective qualification transitions q1—m/z 315.0 > 106.0 (DP = 61.0 V, CE = 57.0 eV) and q2—m/z 315.0 > 255.2 (DP = 61.0 V, CE = 39.0 eV). Under these conditions, the areas of the ion ratios Q/q1 and Q/q2 were 1.6 (RSD = 3.4%) and 2.3 (RSD = 1.9%), respectively. These values agreed with the detections expected for the two and three acquired SRM transitions and confirmed the compliance of their Q/q ratios.

Discussion

Considering the optimum conditions for detection of each dye by SRM, further experiments involved coupling of liquid chromatographic separation with MS/MS detection to analyze 40 μL of the dyes mixture at a concentration of 50 ng mL\(^{-1}\) each under the experimental conditions described in the Experimental section. Figure 2 depicts the respective chromatograms. All the dyes displayed well-defined chromatographic peaks from 13.8 to 21.2 min, which helped differentiate between the analytes on the basis of their retention times. Within this time range, the matrix exerted little effect on the analytes; for example, the TE presented a slightly suppressed signal for most of the target dyes (from 9.0 to 22.0%). As for the receiving waters, all the dyes exhibited signal suppression effects lower than 16.0%.

Figure 2 contains the total ion chromatogram (TIC) and the extracted ion chromatogram (XIC) recorded for each standard disperse azo dye. The optimized SRM conditions enabled successful identification of the analytes (Table I) on the basis of two or three SRM transitions (ion ratios) and their retention time in the chromatogram. Comparison with the chromatograms obtained for the standard samples attested to the identity of the dyes. The results also met the requirements adopted by the European Commission (28). According to this validation guide, variations in the retention time of the analyte and the relative abundance of the SRM transitions should be no more than 2% and around 20%, respectively, as compared with the calibration standards.

SPE optimization

Two hundred and fifty milliliters of water solution of the target samples helped to optimize SPE extraction and to evaluate and validate the extraction efficiency. More specifically, 250 mL of Milli-Q water-TE (tap water) and receiving water (collected near the Water Treatment Station of the city of Araraquara, state of São Paulo, Brazil) spiked with the target dyes at concentrations of 8.0, 25.0 and 50.0 ng L\(^{-1}\) underwent extraction in Strata X cartridges, in triplicate, as described in the “Experimental” section.

Because pH affects how the analyte and the organic matter interact in the investigated waters, it is essential to control pH during the extraction procedure. Sample acidification to pH 3.5 weakened the interaction between the organic material and the analytes. Physical and chemical studies into the properties of analogous dyes have provided mean log \(K_{ow}\) and pK\(_a\) values of around 4.2 and 8.1, respectively (29). Bearing these values in mind, our analytes probably have low water solubility and acidity, which would justify the use of acid medium to ensure efficient interaction of the dyes with the solid phase, reduce interference from other organic compounds and maximize recovery.

Optimization of the solvents used to condition, equilibrate, clean and elute the SPE cartridge revealed that 6 mL of MeOH and 6 mL of water were the best conditions to condition and equilibrate SPE, respectively. The cleaning step removed most of the interferents that could affect extraction efficiency and analyte response during LC-ESI-MS/MS analysis. After SPE, washing of the cartridge with additional 10 mL of water ensured removal of salts that could form adducts with the dyes and diminish their detectability. Finally, cleaning of the cartridge with 10 mL of the dyes mixture in MeOH/Milli-Q water (20:80, v/v) removed unwanted nonpolar compounds.

The nonpolar characteristics of disperse dyes required a blend of high-strength elution solvents, so the optimum solvation system consisted of 5 mL of ACN/MeOH 1:2 and 5 mL of DCM/MeOH 1:1, which provided recovery levels between 70 and 120%. Injection of standard solutions at various concentration levels (six replicate experiments) helped to verify linearity. Statistical calculations attested that the LC-ESI-MS/MS method was precise. According to the \(t\)-test, the calculated and added concentrations did not differ significantly at the 95% confidence level and lay within an acceptable error range. Therefore, the proposed method is potentially applicable to detect and determine disperse dyes in wastewater samples.

Method performance

The first transition aided LC-ESI-MS/MS analysis of various concentrations of the disperse dyes in different matrices (Table II); plot of the ion area counts against the dye concentration afforded the respective calibration curve. All the dyes presented a linear relationship from 2.0 to 100.0 ng mL\(^{-1}\). All the compounds yielded correlation coefficients of 0.992 or higher (Table II).

Table II displays the limits of detection (LODs) and limits of quantification (LOQs) of the equipment and the method (28). The LOD and LOQ of the equipment were 0.5 and 2.0 ng mL\(^{-1}\), respectively; the limits of the method ranged from 2.0 to 8.0 ng L\(^{-1}\), which demonstrated the high sensitivity of the procedure.

Repeated injections of the standard (\(n = 5\)) on the same day (repeatability) and on different days (reproducibility) helped to assess method precision on the basis of the RSD. The intra- and interday analyses afforded RSD values lower than 6 and 13%, respectively, for dyes at concentrations of 25.0 ng mL\(^{-1}\). Evaluation of method accuracy for intra- and interday analyses, in triplicate, gave RSD values of 1.2 and 8.8%, respectively.

As recommended (28), the recovery study in a given concentration zone agreed with our quantification data (Table III). To determine the recovery values for each analyte, we used Milli-Q water, receiving waters and treated water samples spiked with the nine studied dyes at concentrations of 8.0, 25.0 and 50.0 ng L\(^{-1}\) each. Results from triplicate assays pointed to average recoveries greater than 70%, with RSD <20%.

Determination of dyes in environmental samples

Figure 3 shows the LC-ESI-MS/MS chromatograms obtained for the TE sample. Three dyes arose, at retention times \(t_1 = 16.53\) min...
Figure 2. LC-ESI-MS/MS chromatogram of a mixture containing standard solutions of the disperse dyes and extraction of each compound at 50 ng mL$^{-1}$.  

Disperse Dyes in Environmental Water Samples
In July 2012, application of the developed method aided monitoring of dyes in the affluent and effluent waters of the sewage treatment plant of a textile industry located near the Piracicaba River and at points upstream and downstream of the TE (Table IV). The river waters contained dyes at high concentrations, which indicated that the textile industry adopted treatment processes that did not effectively remove these contaminants. Disperse Violet 93 and Disperse Blue 373 occurred in high percentage, which is worrisome because large discharges of these compounds into the aquatic environment harm aquatic life. No dyes appeared upstream of the TE, but the downstream point presented relatively high concentrations of Disperse Blue 373 (84.4 ng L\(^{-1}\)) and Disperse Violet 93 (274.4 ng L\(^{-1}\)), which suggested easy transport of these dyes after they reached surface water.

In both rivers, the concentrations of the dyes Disperse Red 1, Disperse Blue 373 and Disperse Violet 93 ranged from 84.4 to 3453.3 ng L\(^{-1}\). Table IV summarizes all the data described here and evidences exposure of water resources to worrying levels of dyes. Umbuzeiro and coworkers\(^{(8)}\) demonstrated that Disperse Blue 373 and Disperse Violet 93 are positive in the Salmonella/microsome assay; indeed, these authors found that Disperse Blue 373 accounted for 50\% of the mutagenicity detected in the sludge generated by the Drinking Water Treatment Plant in the Piracicaba River (8). Carneiro et al.\(^{(17)}\) also showed
similar concentrations of Disperse Blue 373 and Disperse Violet 93 in the Cristais River.

The environmental studies agreed with those published by other authors that used similar matrices (17, 18). The figures of merits presented herein were comparable to results published for other analytical methods. The LOD and LOQ obtained in this study were better than the limits achieved in previous published methods for the determination of Disperse Dyes in aqueous environmental samples, but accuracy was similar (17, 18).

The actual environmental impact of the levels of dyes detected in this study requires further research involving bioassays and aquatic organisms.

**Conclusions**

Our findings have demonstrated that the LC-ESI-MS/MS method can successfully determine and quantify low concentrations of nine disperse dyes—Red 1, Violet 93, Blue 373, C.I. Orange 1, Orange 3, Orange 25, Yellow 3, Yellow 7 and Red 13—

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**Table IV**

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Sampling</th>
<th>Samples</th>
<th>Disperse Red 1</th>
<th>Disperse Blue 373</th>
<th>Disperse Violet 93</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD (ng L⁻¹)</td>
<td>Mean ± SD (ng L⁻¹)</td>
<td>Mean ± SD (ng L⁻¹)</td>
</tr>
<tr>
<td>Piracicaba River</td>
<td>1</td>
<td>Upstream</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td></td>
<td>2</td>
<td>TE</td>
<td>153.2 ± 8.4</td>
<td>1468.0 ± 256.0</td>
<td>786.6 ± 79.4</td>
</tr>
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<td></td>
<td>Downstream</td>
<td>d</td>
<td>d</td>
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<td>Cristais River</td>
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<td>Upstream</td>
<td>n.d.</td>
<td>n.d.</td>
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<td></td>
<td>Affluent</td>
<td>d</td>
<td>2812.0 ± 416.0</td>
<td>3453.3 ± 685.1</td>
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<td>TE</td>
<td>1126.6 ± 110.3</td>
<td>1474.6 ± 126.1</td>
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<tr>
<td></td>
<td>Downstream</td>
<td>d</td>
<td>84.4 ± 18.0</td>
<td>274.4 ± 23.1</td>
<td></td>
</tr>
</tbody>
</table>

SD, standard deviation; 1, first sampling; 2, second sampling and 3, third sampling; TE, treated effluent; d, detected at concentration level ≥LOD and ≤LOQ; n.d., not detected, n = 3.
simultaneously in environmental samples. The constructed calibration curves present good linearity from 2.0 to 100.0 ng mL$^{-1}$. Detection limits are as low as 2.0 ng L$^{-1}$, which indicates that the method is an excellent means to monitor disperse dyes in environmental samples such as river waters.

As recommended (28) for recovery studies, the limit of quantification corresponds to the lowest concentration of spiked sample recovery that lies between 70 and 120% and has CV < 20%. By studying the method recovery, it was possible to establish the limit of quantification of the dyes in surface water as 8.0 ng L$^{-1}$. The proposed method meets the requirements described by analytical validation guide: it is fast, precise and accurate, and it consumes little solvent.

The limits of detection and quantification are better as compared with other published results (16–18). The accuracy of the developed method is similar to or better than literature values.

Application of the validated method to detect and quantify disperse dyes in TE and receiving waters revealed alarming data regarding the levels of Disperse Red 1, Disperse Blue 375 and Disperse Violet 93. More studies are underway to determine the potential risks of the presence of those dyes in the environment.

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