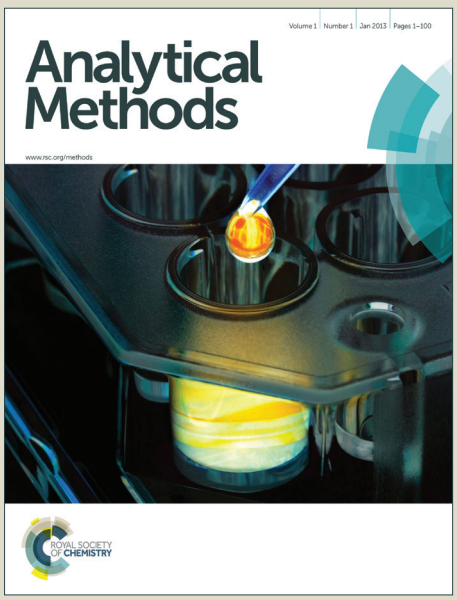
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Using Ionic Liquid Combined with HPLC-DAD to Analyze Semi-Permanent Hair Dyes in Commercial Formulations

Jefferson Honorio Franco[*a], Bianca F. Silva and Maria V. B. Zanoni [a]
[a]Institute of Chemistry-State University "Julio de Mesquita Filho"-UNESP-Avenida Professor Francisco Degni, 55, Quitandinha, CEP: 14800-900, Araraquara-SP, Brazil;
* E-Mail: jeffersonhfranco@gmail.com
* Author to whom correspondence should be addressed; E-Mail: boldrinv@yahoo.com.br; Tel.: +55-16-3301-9500; Fax: +55-16-3322-2308.

ABSTRACT

Strict legislations in different countries have regulated the use of hair colorants and established the limits of dye concentration in cosmetics containing dyestuff. The present work describes a chromatographic HPLC-DAD method to determine the semi-permanent dyes Basic blue 99 (BB99), Acid violet 43 (AV43), Basic brown 16 (BB16), Basic red 76 (BR76) and Basic yellow 57 (BY57) in hair coloring. 1-Buthyl-3-methylimidazolium bis(trifluorometanesulfonyl)imide, BMIm[NTf_2], an ionic liquid (IL) at room temperature, helped to improve the separation and quantification of low levels of basic and acid dyes by HPLC coupled to a diode array detector. The developed method successfully identified the dyes in a commercial semi-permanent hair dyeing formulation and in drinking water. Comparison with an LC-MS/MS technique aided validation of the results.

Keywords: ionic liquid in chromatography; BMIm[NTf2]; HPLC with diode array detection; hair dye.

1. Introduction

Hair coloring is a complex process that has been around for over 2000 years. Nowadays, this method relies particularly on synthetic chemicals involving multiple components. Depending on the fixation that they provide under oxidative conditions, these components are classified as permanent, semi-permanent, and temporary dyes[1]. The dyes employed in semi-permanent and temporary dyeing depend on non-oxidative processes, whereas permanent dyes are based mainly on oxidation reactions.

Permanent hair dyes do not contain dyestuff in their composition. However, they possess colorless precursors and couplers that produce colorful compounds after they penetrate into the hair fiber, a process that involves complex chemical reactions. Because *p*-phenylenediamines and *p*-aminophenols are among the principal precursors, and knowing that they constitute potentially toxic substances[2], permanent dyes have become a matter of major concern[3,4].

Temporary hair colorants consist of water-soluble acid and basic dyes bearing azo or anthraquinone groups. It is possible to remove these dyes by a single application of shampoo. In turn, semi-permanent hair dyes are compounds with low molecular weight composed by acid and basic dyes bearing azo groups, anthraquinones, triphenylmethanes, and nitroderivatives as chromophores. These dyes can resist several washings with shampoo[5]. Their deposition on the structure of the hair takes place via ionic interactions or Van der Waal forces[6]. Such dyes are considered an important group of compunds in the cosmetic area.

Strict legislations in different countries have regulated the use of hair colorants and established the limits of dye concentration in cosmetics containing dyestuff[7]. Most of the analytical methods reported in the literature have focused on the separation and determination of oxidative dye intermediates in permanent hair colorants[8-19]. Despite the importance of

temporary and semi-permanent dyes, few are the analytical methods available to determine them in commercial hair dyeing formulations or in wastewater[20,21]. During the accomplishment of HPLC, dyes strongly adsorb onto the column and are difficult to separate, thereby limiting the application of this method in the routine analysis of basic dyes in hair care products[22,23].

Several researchers have targeted the use of ionic liquids (ILs) at room temperature for chromatographic applications. Addition of an IL may constitute a strategy to improve the chromatographic detection of some analytes[24,25], because ILs usually compete with the basic groups of the analytes for the silanol groups on the stationary phase. Alternatively, ILs can form an ion pair with cationic solutes, to improve the shielding efficiency of the residual silanols. Together, these phenomena improve peak shapes while reducing the chromatographic retention times of the basic analytes.

This work aimed to develop a new chromatographic method to determine semi-permanent dyes, chosen as a model of different chromophores, namely Basic blue 99 (BB99), Acid violet 43 (AV43), Basic brown 16 (BB16), Basic red 76 (BR76) and Basic yellow 57 (BY57) (see the corresponding chemical structures in Figure 1). These dyes have wide application in commercial hair dyeing products. They can be used as hair coloring products without having to be mixed with any oxidizing agent (*e.g.*, hydrogen peroxide). Addition of an IL, like 1-buthyl-3-methylimidazolium bis(trifluorometanesulfonyl)imide, or BMIm[NTf_2], improves the developed method, which can successfully identify dyes in commercial formulations of semi-permanent hair dyeing and drinking water. Application of the LC-MS/MS technique aided validation of the results.

2. Experimental

2.1. Materials and reagents

The dyes BB99, AV43, BB16, BR76 and BY57 as well as the commercial sample HF65 containing all the aforementioned dyes were purchased from LWC Dyes, ARIANOR, São Paulo, Brazil. The sample was analyzed after dissolution in deionized water purified in a MilliQ (Millipore) System. HPLC grade methanol (MeOH) and acetonitrile (ACN) from Merck were used as chromatographic eluents. The IL BMIm[NTf_2] was acquired from Sigma-Aldrich.

2.2. High performance liquid chromatography

HPLC analysis was performed on a chromatograph (Shimadzu, model LC-10AT) equipped with two pumps and an automatic injector (injection volume of 20μL) coupled to a diode array detector (model SPD-M10AVP). The chromatograms were investigated from 200 to 800 nm; the maximum wavelengths selected to analyze the hair dyes BB99, AV43, BB16, BR76 and BY57 were 618, 570, 488, 499 and 383 nm, respectively. The HPLC analysis was performed in a reversed-phase column (Shimadzu CLC-ODS, C18, 25 cm x 4.6 mm x 5 μm, 100 A) connected to a pre-column (Luna Phenomenex, C18, 1 cm x 4.6 mm x 5 μm, 100 A). Before analysis, all the solutions were filtered through a 0.45-μm PTFE filter. The best experimental conditions under the optimized isocratic mode were: mobile-phase acetonitrile/water 65:35 v/v, 2 mL of BMIm[NTf_2] solution 0.040 mol L^{-1}, flow rate of 0.4 mL min^{-1}, and column temperature of 30 °C. The analysis time was 40 min. All the analyses were carried out in triplicate.

The LC-MS/MS measurements were conducted on a tandem quadrupole mass spectrometer (3200 QTRAP, AB Sciex, USA) coupled with a high performance liquid chromatograph equipped with an auto injector (Agilent 1200), a quaternary pump (Agilent 1200), a column oven, and a detector (Agilent 1260 Diode Array); the latter aided acquisition of the spectral data. A heated electrospray ionization interface was used to accomplish the chromatography and extraction procedures as well as the assay validation. The instrument was operated in the positive ion mode for all the dyes, except for AV43, which was analyzed in the negative mode. The source temperature was 550 °C; the ion spray voltage was 5500 V. Nitrogen was the employed gas; the curtain gas was set to 30 psi, the collision gas was set to 12 psi, and gases 1 and 2 were both set to 50 psi. The declustering potential and the collision energy were 60 V and 5 V, respectively, for all the analytes. MRM was used to selectively detect and quantify the analyte. All the experiments were performed in the 50-600 *m/z* range with an input potential of 8 V and trapping in the Q^0-enabled mode. A gradient elution with 0.01% formic acid in water was used in the positive mode. The gradient elution of 0.01 mM ammonium formate in acetonitrile was used in the negative mode. The gradient elution was as follows: from 0 to 0.50 min - 10% ACN, from 0.50 to 15.0 min - 10-70% ACN, from 15.0 to 16.0 min - 70% ACN, from 16.0 to 17.0 min - 70-10% ACN, followed by 3-min conditioning. The injection volume was 20 μL, and the flow rate was 800 μL min^{-1}.

2.3. Instrumentation

The pH was measured on a Digital Brand Gehaka apparatus model PG 2000. The spectrophotometric measurements in the UV-Vis region were carried out on the spectrophotometer Hewlett Packard (HP) model 8453, from 200 to 800 nm, using a 5 mL quartz cuvette (Hellma) with optical path of 1.0 cm. The absorbance spectra in the UV-vis

region were also obtained from the selection of the peaks in the chromatogram itself, in the hydrodynamic mode.

Solid-phase extraction was performed using a Manifold (Vacuum Manifold Processing Station) and Strata-X cartridge 33u polymeric reverse phase with dimension of 200 mg/3 mL (Phenomenex, USA).

2.4. Quantitative analysis

Taking into account the injected volumes, the masses of the hair dyes BB99, AV43, BB16, BR76 and BY57 and the respective analytical curves were obtained by plotting the peak area vs the amount of dye (mol L^{-1}). The concentration of each disperse dye in the environmental sample was obtained by 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.

2.5. Analysis of hair dyes in commercial formulation

The dyes were analyzed in a commercial sample assigned as HF 65 (from Arianor, LCW Dyes, São Paulo, Brazil) containing the hair dyes BB99, AV43, BB16, BR76 and BY57. The sample was prepared following the manufacturer's recommendations. Masses of 1.00 g of the commercial samples containing the dyes and other ingredients as informed in the label (sorbitol, seaweed extract, EDTA, citric acid, benzyl alcohol, and 3-hloro-2-(hydroxypropyl)trimethylammonium chloride, among others) were weighed and placed in a small glass container, diluted with 10 mL of water, and subjected to solid phase extraction in a cartridge containing Strata-X. The best condition for elution was acetonitrile/water 50:50 (v/v). Subsequently, 100 μL of this sample was analyzed by HPLC/DAD using the proposed methodology.

3. Results and discussion

3.1. Effect of ionic liquid on the determination of hair dyes by HPLC-DAD

To achieve the best chromatograms, it was essential to optimize the experimental parameters that affected the chromatographic separation of five temporary hair dyes—BB99 (1), AV43 (2), BB16 (3), BR76 (4), and BY57 (5). The optimal conditions were: a solvent system of acetonitrile/water (45:55, v/v) in the isocratic mode, mobile phase flow rate of 1 mL min^{-1}, injection volume of 20 μL, and temperature of 30°C. Figure 2A depicts the chromatogram of each individual dye (Peaks 1-5). Figure 2B, illustrates the chromatogram of the standard mixture containing all the dyes, being the concentration of each dye in the mixture equal to 8 x 10^{-5} mol L^{-1}, registered under the optimized chromatographic conditions after injection into the HPLC-DAD system.

The chromatogram in Figure 2 A, displayed intense peaks at *t*r = 26.2 min for AV43 (peak 2), *t*r = 16.2 min for BB16 (peak 3), *t*r = 16.5 min for BR76 (peak 4) and *t*r = 11.3 min for BY57 (peak 5). The chromatogram corresponding to BB99 (peak 1) presented three retention peaks: at *t*r = 7.6, 8.5, and 9.8 min, which indicated that the dye sample contained other contaminants. The curve obtained for the mixture of dyes showed ill-defined peaks.

On the basis of the retention time of each dye, monitoring of their UV-vis spectrum in the hydrodynamic conditions of HPLC using a diode array detector coupled to the chromatograph revealed that each spectrum exhibited a set of bands arising from a substituted chromophore system. Maximum absorption arose at 618, 570, 488, 499, and 383 for BB99, AV43, BB16, BR76 and BY57, respectively. Nevertheless, the asymmetric and ill-separated peaks in the chromatogram of the mixture limited quantitative analysis, irrespective of the experimental conditions.

To improve the separation of the five dyes, it was necessary to register chromatograms in the presence of one of the following ionic liquids (ILs): 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide or [BMIm[NTf_2], 1-butyl-3-methylimidazolium tetrafluoroborate or [BMIm-BF_4], and 1-butyl-3-methylimidazolium hexafluorophosphate or [HMIm]PF_6. To this end, we employed a mixture of the dyes (being the concentration of each dye in the mixture equal to 8 x 10^{-5} mol L^{-1}) in acetonitrile/water 35:65 (v/v) containing one of the tested ILs at concentrations ranging from 0.1 x 10^{-2} to 5 x 10^{-2} mol L^{-1}. Chromatographic parameters such as retention time and resolution indicated that BMIm[NTf_2] at 3 x 10^{-2} mol L^{-1} was the best IL to separate the investigated compounds.

Figure 3, Curve B, corresponds to the typical chromatogram achieved for a mixture of the standard solutions containing 8 x 10^{-5} mol L^{-1} of each of the studied dyes in acetonitrile/ water 35:65 (v/v) and BMIm[NTf_2] 3 x 10^{-2} mol L^{-1} at a flow rate of 0.8 mL min^{-1}, T = 30 ^{o}C. The IL elicited slightly different retention times: *t*r = 15.9 min for AV43 (peak 2), *t*r = 20.6 min for BB16 (peak 3), *t*r = 25.2 min for BR76 (peak 4), and *t*r = 34.3 min for BY57 (peak 5). For BB99, the main peak emerged at *t*r = 8.7 min (peak 1), selected to monitor this dye. The individual dyes provided the same chromatographic pattern (Figure 3, Curve A). Addition of the IL afforded better-defined and better-resolved peaks; the initial elution order BB19 > BY57 > BB16 > BR76 > AV43 changed to BB19 > AV43 > BB16 > BR76 > BY57 in the presence of BMIm[NTf_2], in the acetonitrile/water solvent system. These results illustrated that the chromatographic separation is improved due to the following effects: LI cations could be interacting with analyte by formation of ion pair. Thus, decreases the interaction of analyte with the silanol groups of the stationary phase. As the groups nonpolar alkyl of the stationary phase could interact with the quaternary cation of ILs the interaction stationary phase and analyte is decreased and the peak resolution increases. The equilibrium of combined electrical (charge-charge) and hydrophobic interactions with the stationary phase and with ions of the

mobile phase seemed to govern this behavior[26]. ILs contains silanol groups, so these liquids can block the activity of the stationary phase. This should reduce the interaction between the charged dyes, which should translate into improved peak shape and significantly different retention factors. The dual nature of ILs, which bears cations and anions, underlies their interaction with the dyes, thereby affecting the chromatographic results.

The results indicated that the addition of the soluble liquid ionic in water gives a great improvement in the resolution, peak height and shape of the chromatographic peaks since these are dependent on the kinetic interaction between the silanol groups of the stationary phase of the column and the analytes. The majority of the hair dyes has aromatic groups in its structure that are positively charged under the experimental conditions, which causes strong interactions with the residual silanols of the chromatographic column that are negatively charged. The addition of ionic liquid in the mobile phase causes a blocking of the silanols interaction reducing the peak broadening. In addition, the cations of the IL can form ionic pairs with the negatively charged dye molecule and decreases the interaction with the stationary phase which usually disturbs the baseline. These combined effects influence the separation mechanisms occurring on the stationary phase, increases the retention times of the hair dyes and alters the elution order when comparing with the analysis performed in the absence of the ionic liquid. The results thus indicate that ionic liquids can play an important role in the analysis of hair dyes by HPLC/DAD. The strong proton-acceptor properties of these IL can be utilized to suppress the deleterious effects of free silanols on liquid chromatographic separations. The chaotropic character of the anion may introduce ion-pairing with cationic solutes and adsorption on the stationary phase. The hydrophobicity of the cation may further induce stationary phase adsorption.

The chemical structures of the dyes influence on the respective elution on the chromatograms. The dyes BB16, BR76 bear BY57 bears protonated trimethylamide

substituent. The presence of ILs in the mobile phase probably leads to a hydrophobic interactions. This probably increased their retention time in the column, whereas ILs improved the peak shape. Therefore, the IL ions possibly adsorbed onto the C18 surface, while the chaotropic $[NTf_2]^-$ anion associated with a protonated group in the dye, to give less polar ion-pairs. The order in which the dyes eluted—BB99 > BB16 > BR76 > BY57—followed the order in which positive charges were available to interact with the anion of the IL, attesting to the overall strength of the combined solute-stationary phase interactions. The lower elution of AV43 indicated a weaker ion-pair interaction. The majority of the selected dyes were positively charged in the experimental conditions, which caused them to interact strongly with the residual, negatively charged silanols of the chromatographic column.

In the case of Figure 2, without IL peaks were broader and asymmetric due strong interactions between analytes and sylanols group of the stationary phase. In the presence of the ionic liquid this interaction is minimized and the peaks are more symmetric. The retention time of the dyes in figure 2 were different from those seen in Figure 3, because the ionic liquid binds to cationic part of the dye and therefore it interferes with the interaction of the hair dye with the free silanols of the stationary phase and thus the time retention.

Addition of an IL to the mobile phase hindered this dye-column interaction, consequently reducing peak broadening. Concomitantly, the protonated dye may also have interacted with the anion of the IL. The combination of these effects modified the separation mechanism in the stationary phase, which altered the elution order as compared with the analysis performed in the absence of IL.

3.2. Analytical data

We constructed analytical curves (peak area *vs* concentration, in triplicate) for the selected dyes using dye concentrations ranging from 2.97 x 10^{-7} to 1.50 x 10^{-5} mol L^{-1} in acetonitrile/water 35:65 (v/v) containing BMIm[NTf_2] 30 mmol L^{-1}. The flow rate was 0.8 mL min^{-1}, T = 30 ^{o}C. For the chromatographic separations, we employed the experimental conditions optimized by HPLC with DAD detection. We verified good linearity for all the dyes (see Table 1). We subjected the detection limit (L.O.D.), which refers to the smallest level of analyte that provides a measurable response at a 3:1 signal-to-noise (S/N) ratio, to statistical treatment[27]. We estimated the limit of quantification (L.O.Q.), defined as the smallest concentration of the analyte that gives an accurately quantifiable response, at S/N equal to 10:1. The correlation coefficients were close to one, suggesting that it is easy to determine all the dyes in a wide concentration range, with good sensitivity. The chromatograms recorded for each dye at 1 x 10^{-6} mol L^{-1} presented high repeatability, with relative standard deviation lower than 5% for all the measurements (n = 6). The detection limit was around three times lower than that obtained in the absence of the IL (data not shown). The average peak symmetry changed from 0.59 in the absence of IL in the medium to 1.03 in the presence of IL. Therefore, addition of IL to the mobile phase improved peak resolution in the chromatogram. The number of theoretical plates in the presence of an IL should be smaller, as well as the retention peak width. Together, the results indicated that the proposed method is potentially applicable for the analysis of dyes in commercial hair dye formulations and contaminated water samples.

To determine the accuracy and recovery of the method, we spiked tap water samples with standard samples of the dyes at selected concentrations. Figure 4 shows the respective chromatograms obtained after solid phase extraction. The samples were filtered through Millipore MILLEX and submitted to clean-up in a Strata X cartridge (solid phase extraction), as described in the Experimental section. The chromatogram aided monitoring of each dye at

λ = 413 nm. The results are shown in Table 2. We compared the results obtained for this sample with the calibration curve of each dye, constructed in the same experimental condition. Recoveries ranged from 83 to 100%, which attested to the accuracy of the proposed method.

3.3. Determination of hair dye in commercial dyeing samples

We employed the method developed in this work to analyze the commercial dye mixture HF65 under the optimized conditions, after conducting the sample clean-up discussed in the Experimental section. Figure 5 illustrates the chromatograms obtained for the commercial sample (0.01 g L^{-1}). The chromatograms exhibited the peaks relative to the five dyes present in the formulation, confirmed by comparison of both the retention time and UV-vis spectral data with those of authentic samples (Figure 6). Table 3 lists the results from the extrapolation of the calibration curve. It was clear that the dyes described in the product label really existed in the formulation. The amount of each dye varied from 2.0 to 48% (m/m) of the product composition.

To confirm the diagnosis of the main dyes in the commercial dyeing product, we also accomplished LC-ESI-MS/MS analysis of the HF65 formulation. The analysis relied on the attained retention times (t_r), molecular mass (MM), and fragments (Table 4). Chromatograms obtained by ESI (+) MS analysis presented peaks at t_r = 6.4 min, m/z = 415.0 for BB99; t_r = 8.5 min, m/z = 321.3.0 for BB16; t_r = 9.1 min, m/z = 336.4 for BR76; and t_r = 9.4 min, m/z = 336.4 for BY57. Chromatograms obtained by ESI (-) MS analysis displayed peaks at t_r = 9.9 min, m/z = 408.3 for AV43. The presence of the fragments typical of each dye in the MS spectrum (Table 4) confirmed their chemical structure. The method developed here and LC-ESI-MS/MS afforded very close concentration values for each of the dyes in the formulation (Tables 3 and 4), with low standard deviations. The Student *t* test indicated lower values than the critical one (4.30). In conclusion, we have developed an extremely interesting method: it

provided results that did not differ significantly from those achieved with LC-ESI-MS/MS (at 95% confidence level), with the additional advantage that it is more economical.

4. Conclusion

We have developed a method that allows for effective, simultaneous determination of five hair dyes at low concentrations (low detection limits) in commercial hair dyeing samples within an acceptable error range.

Addition of the ionic liquid 1-buthyl-3-methylimidazolium bis(trifluorometanesulfonyl)imide, BMIm[NTf_2], and triethylamine to the mobile phase consisting of 35:65 acetonitrile/water proved to be an excellent strategy to separate the dyes present in the commercial formulation, namely BB99, AV43, BB16, BR76 and BY57 in the C18 HPLC - DAD. The method provided good linearity for all the five dyes, and it was possible to construct calibration curves at low occurrence of dyes. Limits of detection and quantification lay from 10^{-7}, suggesting that the method met with the sensitivity required analyzing commercial formulations and possible wastewater from beauty salon effluent.

The proposed method enabled analysis of dyes in commercial hair dyeing samples (Arianor) after an extraction step in pre-packed Strata-X columns. Chromatographic analysis of the commercial sample Arianor HF 65 indicated the presence of each tested dye in the formulation: 19.0 ± 1.0% of BB99, 4.0 ± 2.0% of AV43, 2.0 ± 1.0% of the BB16, 48.0 ± 1.0% of BR76 and 7.0 ± 1.0% of BY57. The method is simple and economic and could be potentially applicable in water monitoring.

Acknowledgements

The authors are grateful to financial support from CNPq and CAPES and FAPESP (process 2008/10449-7).

References

1 O. J. X. Morel and R. M. Christie, *Chem. Rev.* 2011, **111**, 2537-2561.

2 G. J. Nohynek, F. D. Duche, A. Garrigues, P. A. Meunier, H. Toutain and J. Leclaire, *Toxicol. Lett.* 2005, **158**, 196-212.

3 J. F. Corbett, *J. Soc. Cosmet. Chem.* 1984, **35**, 297-310.

4 C. R. Robbin, *Chemical and Physical Behavior of Human Hair*, 2nd ed.; New York: Springer-Verlag, 1988.

5 J. Lademann, H. Richter, U. Jacobi, A. Patzelt, F. Hueber-Becker and C. Ribaud, *Food. Cem. Toxicol.* 2008, **46**, 2214-2223.

6 J. F. Corbett, *J. Soc. Dyers Colour* 1976, **92**, 285–303.

7 L. H. Wang and S. J. Tsai, *Anal. Biochem*. 2003, **312**, 207-210.

8 N. A. Penner and P. N. Nesterenko, *Analyst* 2000, **125**, 1249-1254.

9 S. P. Wang and H. J. Chen, *J. Chromatogr*. 2002, **979**, 439-446.

10 P. Ghosh and A. K. Sinha, *Anal. lett*. 2008, **41**, 2291-2321.

11 G. Choudhary, *J. Chromatogr*. 1980, **193**, 277-284.

12 H. Tokuda, Y. Kimura and S. Takano, *J. Chromatogr*. 1986, **367**, 345-356.

13 B. Schultz, *J. Chromatogr*. 1984, **299**, 484-486.

14 C. J. Dowle, A. P. Malyan and A. M. Matheson, Analyst 1990, **115**, 105-107.

15 M. C. Gennaro, P. L. Bertolo and E. Marengo, *J. Chromatogr*. 1990, **518**, 149-156.

16 V. Andrisano, R. Gotti, A. M. Pietra and V. Carrini, *J. Liq. Chromatogr. Relat. Technol*. 1994, **17**, 2919-2937.

17 N. Goetz, P. Lasserre, P. Bore and G. Kalopissis, *Int. J. Cosmet. Sci*. 1988, **10**, 63-73.

18 C. Sainthorant, Ph. Morin, M. Dreux, A. Baudry and N. Goetz, *J. Chromatogr*. 1995, **717**, 167-179.

19 C. E. Lin, Y. T. Chen and T. Z. Wang, *J. Chromatogr.* 1999, **837**, 241-252.

20 V. Andrisano, R. Gotti, P. Roveri and V. Cavrini, *Chromatographia* 1997, **44**, 431-437.

21 E. Mariani, C. Neuhoff and C. Villa, *Int. J. Cosmet. Sci*. 1997, **19**, 51-63.

22 E. Mariani, A. Bargagna, M. Longobard and S. Dorato, *Int. J. Cosmet. Sci*. 1994, **16**, 17-27.

23 C. Scarpi, F. Ninci, M. Centini and C. Anselmi, *J. Chromatogr*. 1998, **796**, 319-325.

24 A. M. Calero, V. Pino, J. H. Ayala, V. González and A. M. Afonso, **Talanta** 2009, **79**, 590-597.

25 T. L. Mescoloto and M. V. B. Zanoni, *Molecules* 2012, **17**, 7961-7979.

26 Y. Wang, M. Tian and K. H. Row, *J. Int. Mol. Sci*. 2009, **10**, 2591-2610.

27 L. R. Snyder, J. J. Kirkland and J. L. Glajch, *Practical HPLC Method Development*. 2nd ed.; New York: John Wiley & Sons, 199

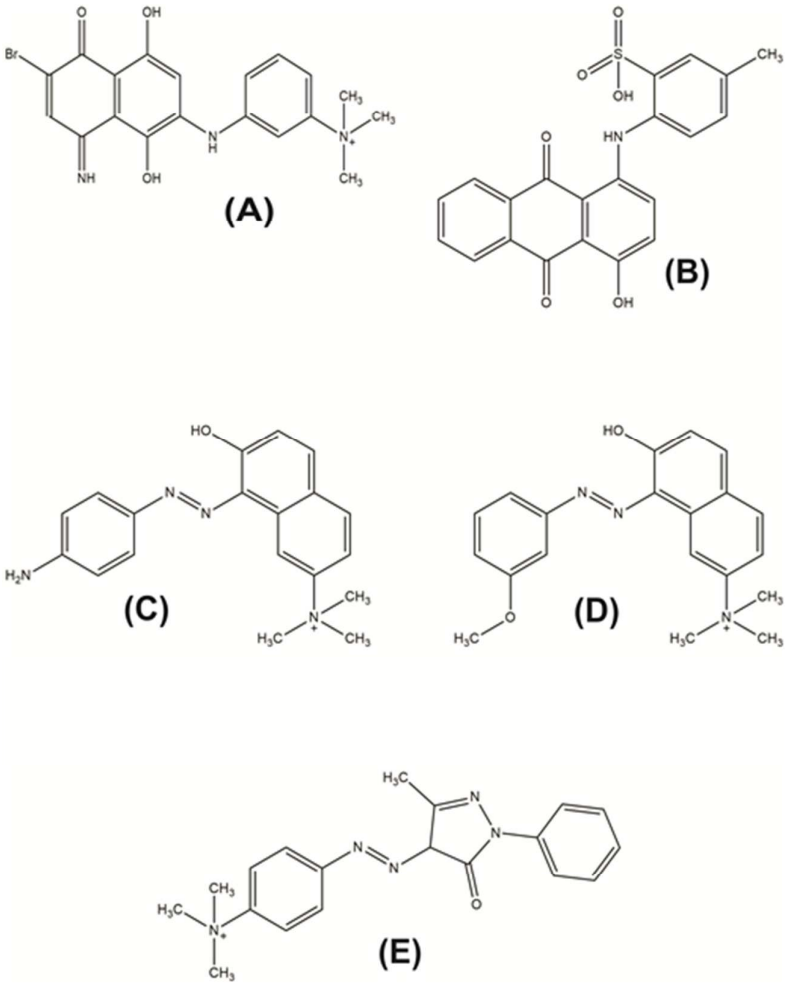


Figure 1. Chemical structures of (A) basic blue 99 dye (BB99), (B) acid violet 43 (AV43), (C) basic brow 16 (BB16), (D) basic red 76 (BR76) and (E) basic yellow 57(BY57).

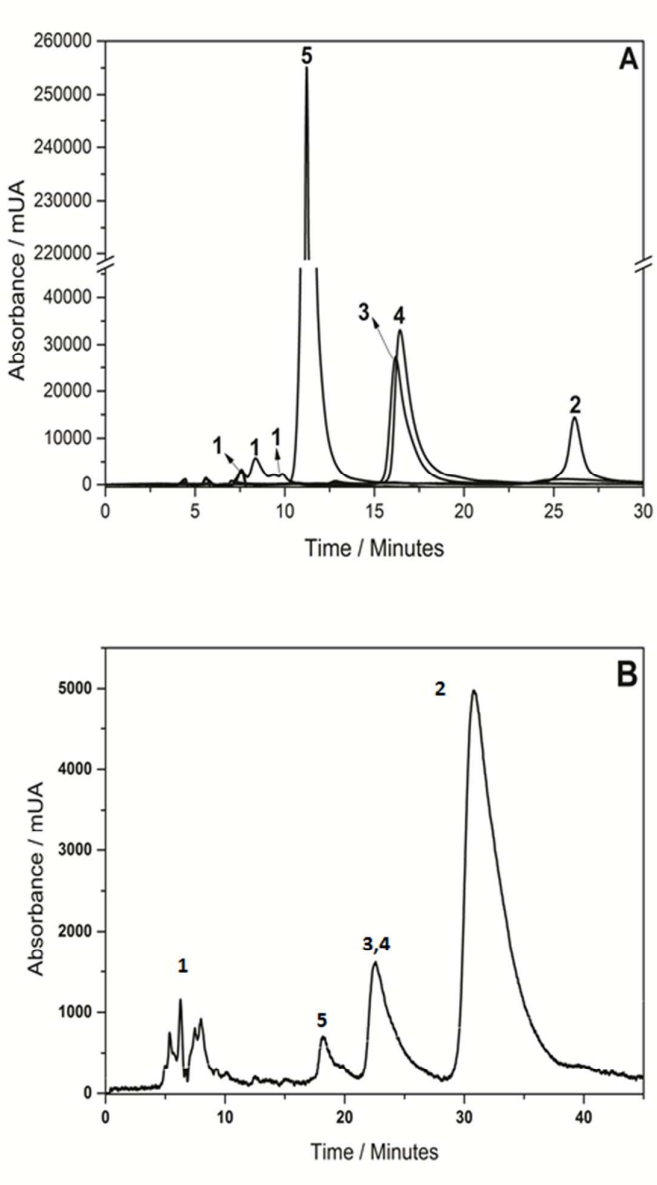


Figure 2. HPLC/DAD chromatograms obtained for 20 μL (**A**) of standard solution of $8x10^{-5}$ mol L^{-1} of the hair dyes: (1) BB99, (2) AV43, (3) BB16, (4) BR76 and (5) BY57, respectively and their mixture (**B**). Mobile phase: ACN/phosfate buffer (45:55, v/v). T: 40ºC, flow-rate: 1.0 mL min^{-1}; C18 phase. Wavelength of standard dyes: λ= 383 nm; 482 nm; 499 nm; 523 nm e 618 nm. Wavelength of mixture: λ= 413nm.

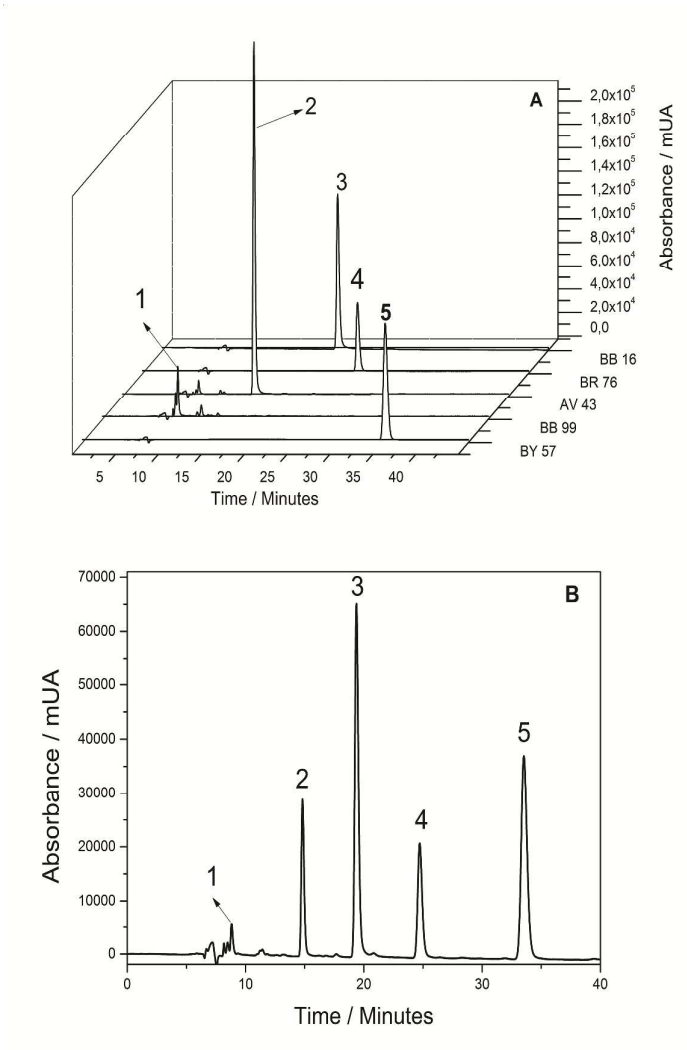


Figure 3. Efect of ionic liquid on the HPLC/DAD chromatograms obtained for 20 μL (**A**) of standard solution of $8x10^{-5}$ mol L^{-1} of the hair dyes: (1) BB99, (2) AV43, (3) BB16, (4) BR76 and (5) BY57, respectively and their mixture (**B**). Mobile phase: ACN/water + 40 mM BMIm[NTf_2] 35:65 (v/v). T: 40ºC, flow-rate: 0.4 mL min^{-1}; C18 column. Wavelength of standard dyes: λ= 383nm; 482nm; 499nm; 523nm e 618nm. Wavelength of mixture: λ= 413nm.

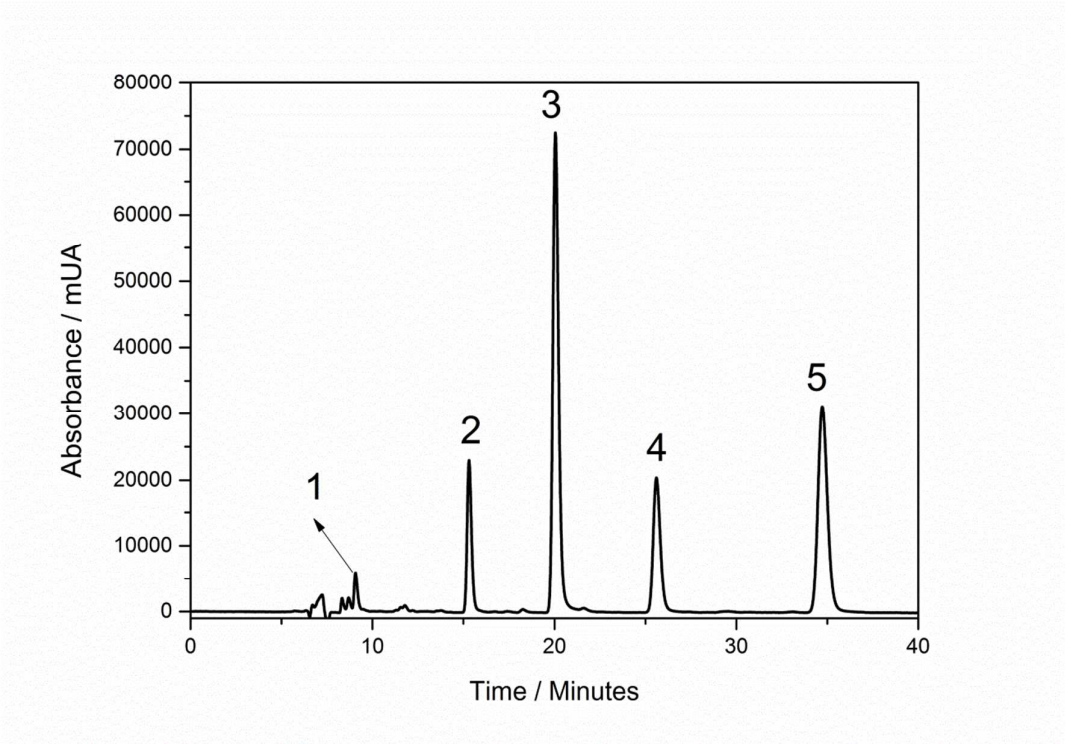


Figure 4. HPLC/DAD chromatogram obtained for the mixture of 1: BB99, 2: AV43, 3: BB16, 4: BR76 and 5: BY57 dyes at concentration of $8x10^{-5}$ mol L^{-1}, extracted in 50:50 ACN/H_2O and eluted with ACN/ H_2O + 40 mM BMIm[NTf_2] 35:65 (v/v). T: 40ºC, flow-rate: 0.4 mL min^{-1}; C18 column. λ= 413nm.

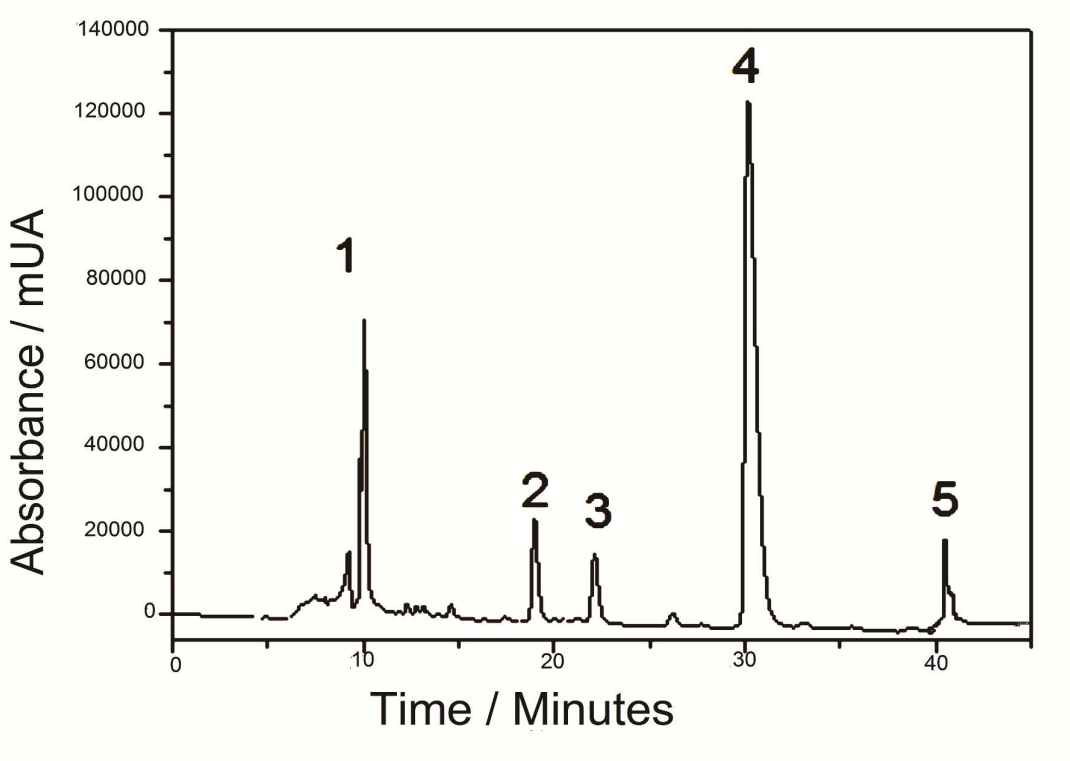


Figure 5. HPLC/DAD chromatograms obtained for a commercial hair dye sample assigned as HF 65 (Arianor, LCW Dyes, São Paulo, Brazil). ACN/ H_2O + 40 mM BMIm[NTf_2] 35:65 (v/v). T: 40 ºC, flow-rate: 0.4 mL min^{-1}; C18 column. λ= 413 nm. 1: BB99, 2: AV43, 3: BB16, 4: BR76 and 5: BY57.

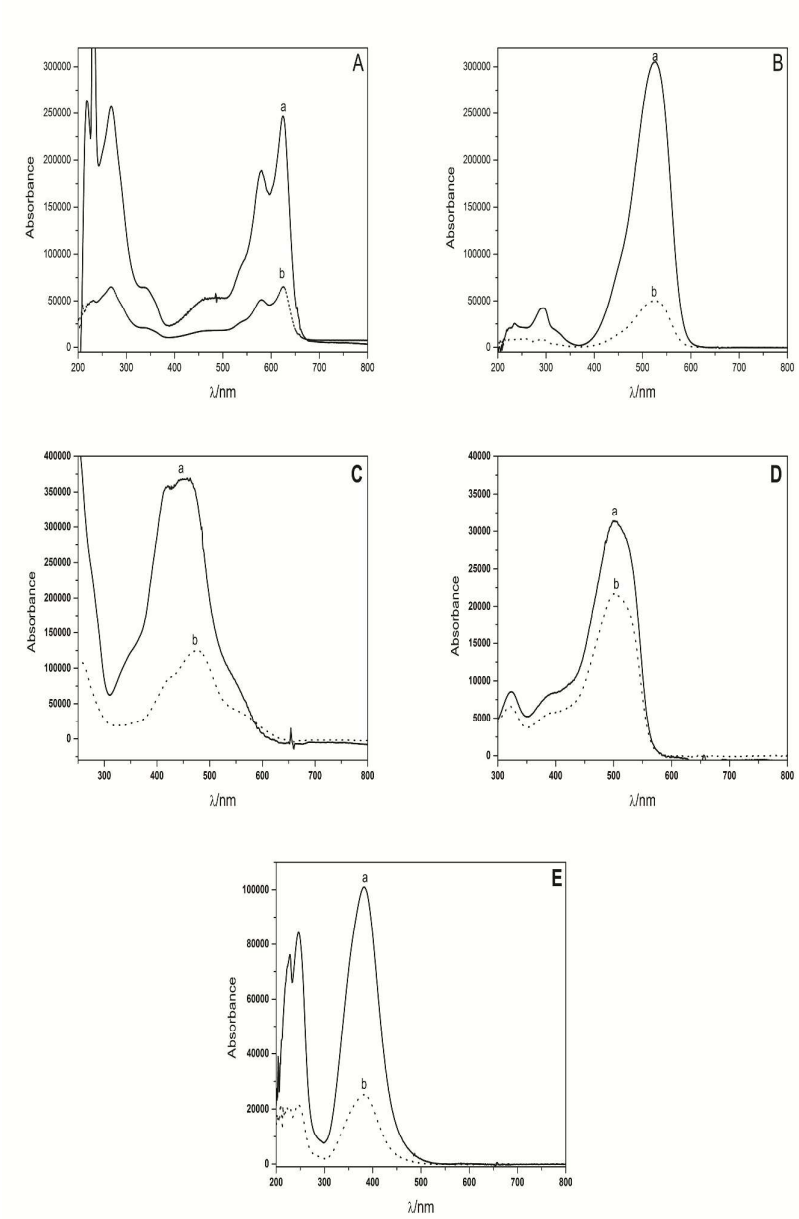


Figure 6. (a) UV-Vis spectra obtained from hydrodynamic chromatograms corresponding to each retention time of (A): BB99, (B): AV43, (C): BB16, (D): BR76 and (E): BY57, respectively for standard dye sample (a) and commercial sample (b).

Table 1. HPLC-DAD analytical parameters obtained for determination of hair dyes. Flow rate: 0.4 mL min^{-1}, mobile phase: ACN/water + 40 mM BMIm[NTf_2] 35:65 (v/v). T: 40ºC, flow-rate: 0.4 mL min^{-1}; C18 column.

Dye	A	B	R	L.O.D.	L.O.Q.	C	D
BB99	133.295.70	1.91×10^{10}	0.9912	2.98×10^{-7}	3.66×10^{-7}	7.570	8.710
AV43	-78.208.70	5.34×10^{10}	0.9971	2.71×10^{-7}	3.21×10^{-7}	26.23	15.86
BB16	148.466.0	2.92×10^{10}	0.9905	0.53×10^{-7}	1.08×10^{-7}	16.15	20.58
BR76	65.552.90	2.01×10^{10}	0.9925	2.91×10^{-7}	3.06×10^{-7}	17.48	25.17
BY57	-10.277.60	2.05×10^{10}	0.9915	1.09×10^{-7}	1.41×10^{-7}	11.29	35.30

*A: linear coefficient; B: Slope; L.O.D.: Limit of Detection (mol L^{-1}); L.O.Q.: Limit of Quantification (mol L^{-1}); R: correlation coefficient; C: retention time without IL; D: retention time with IL.

Table 2. Percentage of dye recovery in tap water fortified with BB99, AV43, BB16, BR76, and BY 57 dye submitted to solid phase extraction with Strata X cartridges.

Dye	A	B	Rec
BB99	7.50×10^{-6}	7.51×10^{-6}	100.0 ± 1.0
AV43	2.93×10^{-5}	2.52×10^{-5}	86.0 ± 2.0
BB16	8.65×10^{-6}	7.18×10^{-6}	83.0 ± 2.0
BR76	1.32×10^{-5}	1.16×10^{-5}	88.0 ± 1.0
BY57	1.96×10^{-5}	1.78×10^{-5}	90.0 ± 1.0

*A: Added concentration (mol L^{-1}); B: Concentration after extraction by SPE; Rec: % of recovery.

Table 3. Determination of hair dyes in the commercial hair dyeing formulation HF 65 Arianor by HPLC/DAD under the following analytical conditions: 1.0 g of commercial dye in 10 mL of water, ACN/water + 40 mM BMIm[NTf_2] 35:65 (v/v). T: 40ºC, flow-rate: 0.4 mL min^{-1}; C18 column; λ = 413 nm (measured in triplicate).

A	C_{found} (mol L^{-1})	C_{found} (mg/g)	% of dye Proposed method	% of dye LC-MS-MS method
BB 99	4.33×10^{-5}	23.46	19.0 ± 0.98	22.0 ± 1.98
AV 43	8.81×10^{-6}	4.56	4.0 ± 1.05	3.7 ± 1.56
BB 16	5.86×10^{-6}	2.51	2.0 ± 0.99	2.5 ± 1.92
BR 76	1.31×10^{-4}	58.40	48.0 ± 0.96	57.0 ± 2.96
BY57	1.99×10^{-5}	8.88	7.0 ± 0.97	5.4 ± 2.3

*A: Hair dyes; B: Concentration found in commercial sample (mol L^{-1}); C: Mass of the dye in commercial dye (commercial dye 1g/120g); D: Percentage of hair dyes in commercial dye (%).

Table 4. Analysis of hair dyes in the commercial hair dyeing formulation.HF65 Arianor by LC/MS-MS.

Hair dyes	Retention time (min)	Precursor ions (*m/z*)	Fragment ions (*m/z*)
BB99	6.4	415.0	402; 358
AV43	9.9	408.3	393.3
BB16	8.5	321.3	306.2; 291.2; 276.2
BR76	9.1	336.4	321.3
BY57	9.4	327.4	321.3; 150.0