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Analysis of Early Synthetic Dyes with HPLC-DAD-MS

An important database for analysis of colorants used in cultural heritage

By

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Preamble

This work is focused in the analysis of 62 early synthetic dyes from the second half of the nineteenth century (1850-1900), from the Smithsonian's Helmut Schweppe Collection. The main purpose of this master thesis was to develop a powerful HPLC-DAD-MS^n database in the Department of Conservation and Restoration, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (DCR, FCT-UNL) in collaboration with Centro de Química Estrutural do Instituto Superior Técnico (CQE/IST), under the framework of the Project: *Unveiling the secrets of molecules with colour and history,* POCI/ QUI/099388/2008 and REDE/1502/REM/2005. With this synthetic dyes database it will be possible to identify and characterize early synthetic dyes in works of art, as for example in textiles and ethnographic objects, just to name a few.

For the first time, the early synthetic dyes were analyzed and characterized with HPLC-DAD-MS^n as well as identified in a Persian carpet (T107) from the 20^{th} century. The Persian Carpet, from the Gulbenkian Museum was performed by an Armenian restorer, who did several stitch restorations in other textiles from Gulbenkian collection. Therefore the results obtained, can contribute for questions related with textiles date, identification of old restorations in the Gulbenkian collection, as well as, contribute to conservation and restorations treatments.

The thesis is elaborated according with the guidelines of Journal of Chromatography A and the references system of Studies in Conservation as recommended by the master thesis guidelines in conservation and restoration. This work will be submitted to Journal of Chromatography A [1]. Preliminary results of this work were presented in the 5º Encontro Nacional de Cromatografia [2].

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High performance liquid chromatography with diode array detector and coupled mass detection analysis of early synthetic dyes

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Abstract

The analysis of 62 early synthetic dyes from the Smithsonian's Helmut Schweppe Collection with High Performance Liquid Chromatography – Diode Array Detection – Mass Spectrometry (HPLC-DAD-MS^n) was performed. Several chromatographic and mass parameters were optimized in order to characterize the 62 dyes. This approach was also applied to selected natural dyes because early synthetic dyes were known to be dyed together with dyes from natural biological sources. Prior to the HPLC-DAD-MS^n analysis, soft extraction methods were tested on dyed textiles.

In the present study it was possible to characterize the 62 dyes from 11 chemical families as well as selected natural dyes using the same methodology. Also the soft extraction methods were successful in both synthetic and natural dyes recovery.

This HPLC-DAD-MS^n database was compared with samples from a Persian carpet, from 20th century. It was possible to conclude that the carpet was dyed with synthetic dyes. The yellow and beige colours were obtained with a mixture of azo flavine 3R (CI 13090) and orange IV (13080,) and the red colours were obtained with scarlet n for silk (CI 15635) and cotton scarlet (CI 27290). The blue colours were obtained with synthetic dyes probably from the tryarilmethane family. The green colour was obtained with a mixture of the yellow and blue synthetic dyes mentioned previously.

Keywords: Early synthetic dyes, HPLC-DAD-MS^n, TEA , azo flavine 3R, orange IV, scarlet N for silk, cotton scarlet

1. Introduction

Before 1856, the only resource for dyeing textile fabrics was natural dyes [3]. Ancient dyers, who were highly skilled and trained, could produce many rich colours, but there were particular shades that were extremely difficult to produce, or at least in a form that was durable. As a result, some natural dyes were costly and thus an important symbol of status and wealth [4-6]. With the discovery of the first manufactured purple synthetic dye in 1856 by Perkin as well as other synthetic dyes obtained from coal-tar chemicals in the following years, an unlimited range of bright, fast and inexpensive colours became (Appendix I, p. 24) available to a large public. New colours were introduced and as a result, textiles and fashion culture were irrevocably transformed.

The chemist Helmut Schweppe collected the 65 most used early synthetic dyes between 1850 and 1900 on his work about textiles dyeing Reference Collection DYE 16850-16915, "Important Early Synthetic Dyes" and dyed fibers "Colourings of Early Synthetic Dyes" [7] (Appendix I, p. 24). The Schweppe collection represents a challenge in terms of analytical separation and characterization due to the chemical diversity of the dyes found in this collection. In this collection, textiles samples were dyed with 11 different chemical dye families (azo, triarylmethane, xanthene, anthraquinone, azine, nitro, diphenylmethane, quinoline, thiazine, aminoketone/lactone and indigoid), table 2 (Appendix I, p.24) ranging four dyeing methods of application (acid, basic, direct or mordant). Dyestuffs, usually contain several chromophores and the high-performance liquid chromatography (HPLC) coupled with the diode array detection (DAD) technique is a separation method that allows the identification of colouring matters and their UV-Vis absorbances [6]. Marteen Van Bommel *et al* [8] analysed this collection with HPLC-DAD and 3D fluorescence recommending the utilization of two elution gradient HPLC systems, one for acid dyes and a second one for basic dyes. Since it is unknown whether a textile sample is dyed with an acid or a basic dye, the both systems for the HPLC analyses should be considered and if the sample size allows a twofold analysis. In this work a single HPLC system is proposed, being possible to use a single textile sample for dyes identification. Moreover, the single system proposed is compatible with mass spectrometry analysis. In the previous work [8] phosphoric acid and tetra butyl ammonium hydroxide (TBA), non volatile solvents were used, excluding their application in HPLC-DAD-MS^n analysis.

The utilization of mass spectrometry is fundamental in the characterization of early synthetic dyes, once the identification of components in complex mixtures of dyes requires a more discriminating tool than UV-Vis spectroscopy [9-10]. This is particularly important in dyes with similar structures and UV-Vis spectra as some early synthetic dyes, which can be easily discriminated with mass spectroscopy. Mass spectroscopy besides providing the molecular weight of the dye molecule, sometimes yields additional structural information based on fragmentation patterns [10-11]. The most used ionization technique for liquid-chromatography-mass spectrometry (LC-MS) is the "soft ionization" which produces primarily molecular species with little fragmentation [12]. This fragmentation can be seen through tandem mass spectrometry (MS-MS) where an ion of interest is isolated on a first stage, followed by other generated fragmentation ions, on a second stage. [12] Electrospray ionization (ESI) is a soft ionization technique, taken at atmospheric pressure, where the dissolved analyte pass through a metal capillary that is maintained at high voltage and where is dispersed by electrospray into a fine aerosol. Due to the high voltage, highly charged droplets are formed and dissolved as they pass

through the atmospheric-pressure region of the source towards a counter electrode. A drying gas, usually nitrogen, is continually passing into the spray region to assist the desolvation. When the droplets are pumped into the source of mass spectrometer, fragmentation ions can be obtained. [12]. ESI technique is the most used for dyes with an ionic or very polar character [15], being the most suitable for the Schweppe collection.

The characterization of the Schweppe collection will be compared with a Persian carpet from the beginning of 20th century (T107), from Gulbenkian Museum. This carpet was made by an Armenian weaver apparently with natural materials (dyes and fibres), who did stitch restorations in other textiles from Gulbenkian collection. The identification of the dyes used in this carpet with the HPLC-DAD-MS^{n} database can reveal important contributions in questions related with textiles date, originality, as well as conservation and restoration issues.

2. Experimental

2.1 Samples

2.1.1 Helmut Schweppe dyeing Reference Collection DYE 16850-16915

Standards of the 62 dyestuffs were acquired from different suppliers (Ciba, Riedel, etc.) in the 80s by Museum Conservation Institute - Smithsonian Institution and are listed in table 4 (Appendix III, p.30). From those 62 standards, 14 samples (CI 13065, 15620, 15635, 16045, 16050, 16100, 16185, 16255, 16290, 18050, 18055, 21000, 26900 and 27290) were wool textiles dyed according H. Schweppe [7] (Appendix III, p.30) Three natural dyes, weld, cochineal and indigo were acquired from Extrasynthèse (Genay, France). A mauve salt labeled as the "original mauveine prepared by Sir William Henry Perkin in 1856" was obtained from the Science Museum.

2.1.2 Persian Carpet 20th century (T107)

9 samples from the Persian carpet, T107 (1 blue: L= 20.94 $\pm$ 0.12; a*= -2.12 $\pm$ 0.02; b*= 5.54 $\pm$ 0.13, 2 yellow: L= 50.66 $\pm$ 0,08; a*= 3.87 $\pm$ 0; b*= 26.19 $\pm$ 0.02, 1 green: L= 35.68 $\pm$ 0.07; a*= 1.21 $\pm$ 0.04; b*= 22.85 $\pm$ 0.07 and 4 red: L= 18,30 $\pm$ 0,10; a*=17, 86 $\pm$ 0,03; b*= 6,55 $\pm$ 0,11) were collected for HPLC-DAD-Msn analysis (Appendix II, p.27). It was used for HPLC-DAD-MS^{n} 2 textile samples from each colour weighting *ca*. 0.2-0.6 mg.

2.2 Sample preparation and extraction methods

2.2.1 Pure dyestuffs

Prior to HPLC-DAD analysis of the pure dyestuffs, several solvent proportions were tested in the dissolution of the fuchsine standard: **(a)** 100% H_2O; **(b)** H_2O: CH_3OH (80:20, v/v). The best result acquired was applied to all the pure dyestuffs and textile samples.

0,2 mg of the pure dyestuff were dissolved in 4 mL of H_2O: MeOH (80:20,v/v), with exception of Indigo, which was dissolved in DMF [13]. 25µL of the final solution was used for HPLC-DAD analysis.

2.2.2 Textile samples

Four different extraction solutions were tested previously in wool fibers dyed with cloth scarlet (CI 26900), prior to the HPLC-DAD-MS^n analysis of historical dyed-textile samples: **(a)** *Formic acid method* - HCOOH: CH_3OH (5: 95, v/v) [14]; **(b)** *HCl method*- 37%: CH_3OH: H_2O (2:1:1, v/v/v) [14]; **(c)** *Oxalic acid method* - $C_2O_4H_2$ (0,2M): C_3H_6O: CH_3OH: H_2O (0,1: 3: 3: 4, v/v/v/v) [14]; and **(d)** *TFA method*- TFA 2M [15]. The analyses were performed in three replicates.

The extraction was carried out as follows: a small sample of thread was extracted with the extraction solution in 1.5ml eppendorfs for 30 min, in 60ºC water bath, with constant agitation. After extraction, each extract was dried in a vacuum system, where the resulting dry residues were reconstituted with 50 µl H_2O: MeOH (80:20,v/v). The best result was applied to all textile samples (Appendix III, p.30)

2.3 Conditions of analysis

2.3.1 HPLC-DAD

The dye analyses were performed in a *Thermofinnigan Surveyor* HPLC-DAD system with a *Thermofinnigan Surveyor* PDA 5 diode array detector (Thermofinnigan, USA), an autosampler and a pump. The separations were performed in Zorbax Eclipse Plus C18 (Agilent, USA) with 5µm particle size column (2.1 mm x 150 mm). The column was kept at controlled temperature (35ºC). The samples were injected onto the column via a Rheodyne injector with a 25µl loop. A solvent gradient of (A) TEA pH= 6,4 3mM and (B) pure methanol adapted from [10] was used at a flow rate of 0.3 mL/min; 0-0,01min A:B (95:5) isocratic, 5 min A:B (90:10) isocratic, 10 min A:B (70:30) isocratic, 15 min A:B (50:50) isocratic, 30 min A:B (45:55) isocratic, 35 min A:B (30:70) isocratic, 45-55 min A:B (5:95) linear, 60-70 min A:B (95:5) linear.

2.3.2 HPLC-DAD-Ms^n

The analyses of dyes were performed on a LC-MS with ProStar 410 autosampler, two 212-LC chromatography pumps, a ProStar 335 diode array detector and a 500-MS ion trap mass spectrometer with an electrospray ionization (ESI) ion source (Varian, Palo Alto, CA, USA). Data acquisition and processing were performed using Varian MS Control 6.9 software. The separations were carried out using a Zorbax Eclipse Plus (Agilent, USA) with 5 µm particle size column (2.1 mm x 150 mm). The column was kept at controlled temperature (35ºC). The samples were injected onto the column via a Rheodyne injector with a 20 µL loop. The gradient adapted from and described in 2.3.1 was used at a flow rate of 0.2 mL/min. The mass spectra were acquired in negative and positive mode depending on the structures. The optimized parameters were as follows: ion spray voltage, ± 4,8 kV; capillary voltage, 20 V; RF loading 80%. Nitrogen was used as nebulising and drying gas, at a pressure of 35 and 15 psi; drying gas temperature, 300ºC. The multistage MS (MS^n) spectra were obtained with an isolation window of 2.0 Da, excitation energy values of 0,9 to 1,5 V and an excitation time of 10 ms.

3. Results and discussion

With the HPLC-DAD-Ms^n system studied it was possible to separate and characterize 62 early synthetic dyes from the Helmut Schweppe collection. The combination of HPLC-DAD and mass spectrometry was successful in separating and characterizing compounds from different chemical

families as well as distinguishing compounds with a similar structure. For example, Orange II (CI 15510) and Croceine Orange G (CI 15970) with a similar retention time and UV-Vis spectrum displayed different mass spectra which allowed its differentiation, for more details, see next sections.

3.1 HPLC-DAD-MS^n conditions

3.1.1 Sample preparation and extraction methods

Better HPLC separations of fuchsine standard were achieved with H_2O: CH_3OH (80:20,v/v), figure 1b, than using only H_2O, figure 1a, and this result was applied to all the standards and textiles analysed.

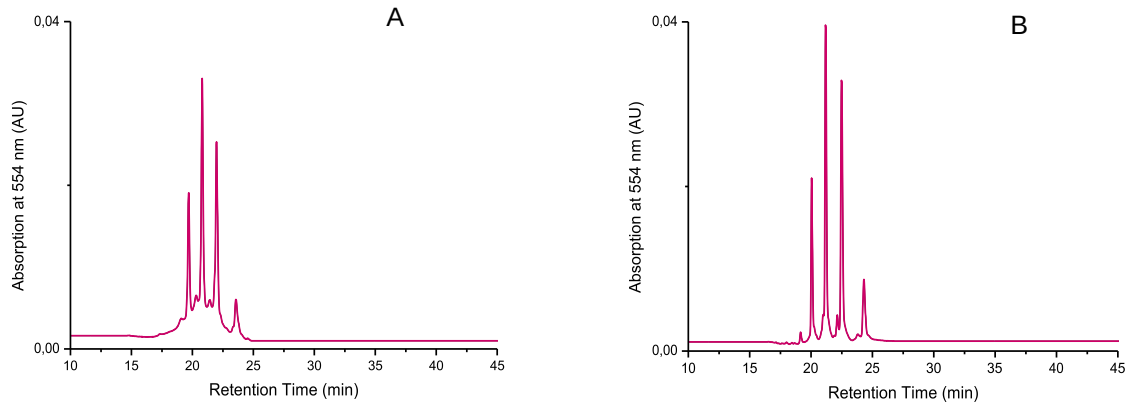

Figure 1– HPLC-DAD chromatogram of fuchsine standard (CI 42510), acquired at 554 nm, dissolved in: A) 100% H_2O; B) H_2O:MeOH (80:20,v/v).

Contrarily to natural dyes [10], early synthetic dyes usually are not affected by the hydrochloric treatment [8]. Nevertheless, it is known that textiles might have been dyed with both synthetic and natural dyes [8]. Therefore, the HCl method should be avoided in the extraction of synthetic dyes once it can promote the decomposition of glycosidic chromophores from natural dyes [10]. Three soft extraction methods already used in natural dyes extraction were tested for the first time in synthetic dyes, namely the formic acid [10], oxalic acid [16] and TFA method [15]. The oxalic acid and TFA extraction were the most efficient methods for cloth scarlet, an azo dye, with similar extraction yields to HCl method [14], figure 2. The TFA method with the higher extraction yield as well as the best signal-to-noise ratio (S/N) in HPLC-DAD-Ms^n for several natural dyes [15] was applied in the textiles samples from the Persian Carpet T107.

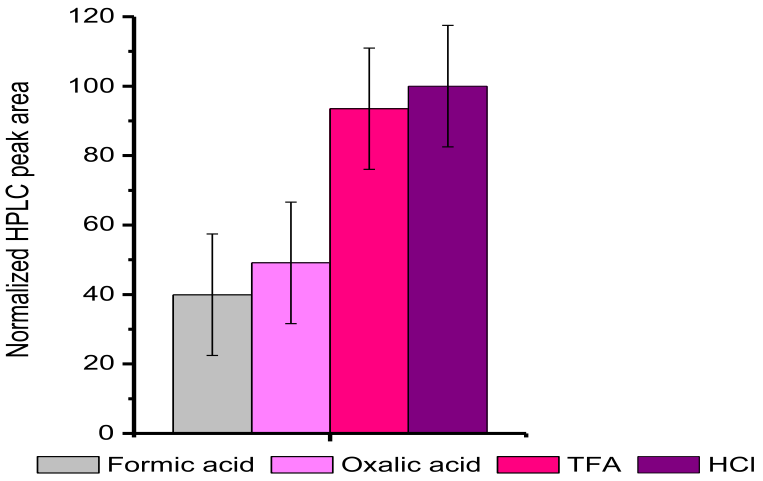

Figure 2 - HPLC peak areas measured for cloth scarlet (CI 26900) extracted from dyed fiber with formic acid, HCl, oxalic acid and TFA method and normalized to the highest value (taken as 100%) of the four methods.

3.1.2 Optimization of elution method

The triethylamine (TEA) solvent has been successfully used as an ion-pair reagent for analysis of azo dyes with HPLC-DAD-ES-MS^n, besides tributylammonium acetate (TBAA) and dihexylammonium acetate (DHAA), being TEA a good choice for molecules with molecular weights between 350 and 1220 [17]. The TEA in the mobile phase often improves the retention and separation of mono- and disulfonic aromatic acidic dyes and in some cases, dyes containing more than two sulfonic acid groups [19]. In the Schweppe collection, 52% of the dyes contain sulfonic groups in their composition. Therefore, in this work, a gradient using TEA and MeOH as mobile phase adapted from [18] was applied not only in the separation of dyes with sulfonic groups, but also dyes with other functional groups as nitro, carboxylic acids, etc. The time of analysis was increased as well as the percentage of MeOH relatively to the original gradient [18], which allowed the analysis of apolar dyes as diamond green G (rt= 39.23 and 40.60 min.), crystal violet (rt= 39.83 min.), victoria blue B (rt=42.87 and 43.24 min.) and rhodamine B (rt=38.86 min.), among others. With this gradient 62 dyes from 11 different chemical families were reasonable separated, table 4 (Appendix III, p.30). Complex mixtures were also resolved with this method, figure 3. Only azo flavine 3R (CI 13090), light green SF bluish (CI 42075), methyl blue (CI 42780) and alizarin (CI 58000) displayed poor chromatographic resolution. In order to increase the chromatograms resolution of these dyes, they were acidified with 1 drop of H_2O: CH_3OH: $H_2O/HClO_4$, pH=2 (50:20:30, v/v/v), figure 4. Natural dyes (weld, cochineal and indigo) were also well separated with this gradient, table 4 (Appendix III, p.30).

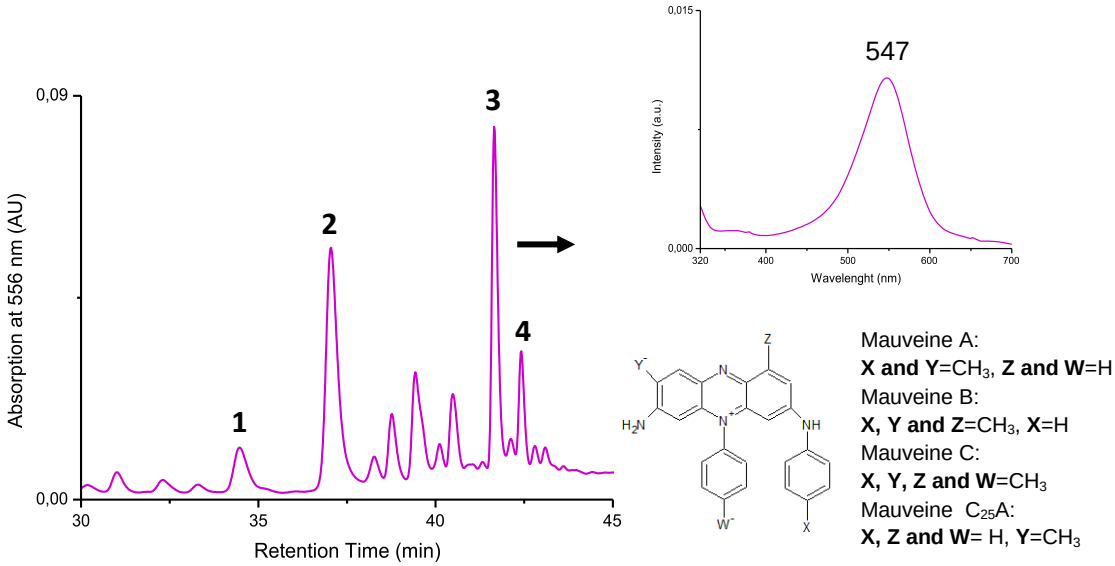

Figure 3 – HPLC-DAD chromatogram of a mauve– dye standard from the Science Museum acquired at 556 nm, which is composed by a complex mixture of several chromophores, all containing the 7-amino-5-phenyl-3-(phenylamino)phenazin-5-ium core with different number of methyl groups in their molecular structure. The majour compounds identified correspond to the numbered peaks: 1 – two C_{25} isomers ($[M]^+$ *m/z*=377), 2 – mauveine A ($[M]^+$ *m/z*=391), 3 – mauveine B ($[M]^+$ *m/z*=405), 4 – Mauveine C ($[M]^+$ *m/z*=419). For more details see next sections.

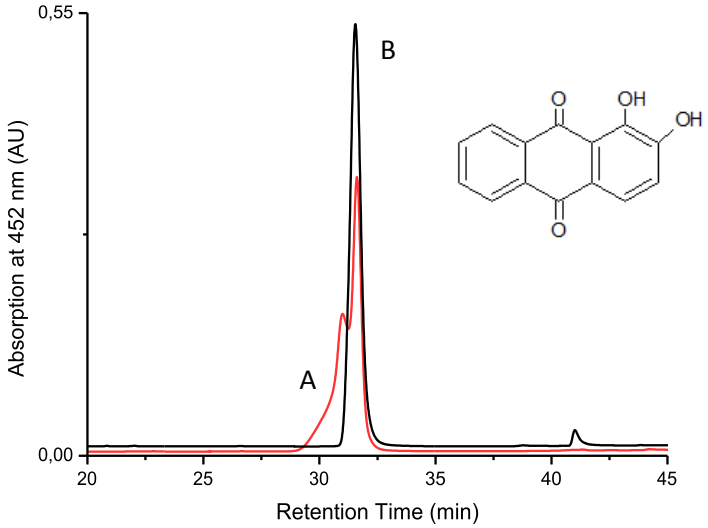

Figure 4– HPLC chromatogram of alizarin standard (H2O:MeOH, 20:80 (v/v)) acquired at 452nm. A – Before acidification with one drop H_2O: CH_3OH: $H_2O/HClO_4$, pH=2 (50:20:30, v/v/v), B – After acidification.

3.1.3 **Optimization of the mass spectrometry conditions**

Electrospray ionization (ESI) is the most suitable ionization technique for dyes with an ionic or very polar character and it is usually used for anionic dyes as nitro and azo, with sulfonic groups. The series of deprotonated molecules enables an easy determination of molecular weights and the number of acid groups.

Non-polar and cationic dyes as chrysoidine (CI 11270), auramine O (CI 41000), fuschine (CI 42510), methyl violet (CI 42535), crystal violet (CI 42555), victoria blue R (CI 44040), victoria blue B (CI 44045), rhodamine 6G (CI 45160), rhodamine B (CI 45170), uranine (CI 45350), mauveine (CI 50245) and methylene blue (CI 52015) were measured in positive mode, with the characteristic molecular adduct $[M+H]^+$ ion [19]. All the other dyes were analyzed in negative mode.

3.2 Results and discussion

3.2.1 Nitro dyes

The nitro dyes were efficiently separated using the HPLC gradient with TEA. As expected [17], the retention time of nitro dyes in the mobile phase decreases with the increase of sulfonic acid groups in the dye molecule. As a result, naphtol yellow (CI 13016) is eluted before martius yellow (CI 13015) and even picric acid (CI 10305), table 4 (Appendix III, p.30)

The negative ion ESI-MS spectrum of nitro dyes exhibit the $[M-H]^-$ ion as the base peak for picric acid and martius yellow, table 4 (Appendix III, p.30). For naphthol yellow, a loss of 45u was observed corresponding to the loss of two Na^+ ions: $[M-2Na+H]^-$. The tandem mass spectrometric (MS/MS) experiments for the picric acid and martius yellow depronated molecules [M-H]-, exhibits the effect of nitro groups [19] by the neutral losses of NO (ion at *m/z* 198 for picric acid and ion at *m/z* 203 for martius yellow) and NO_2 (ion at *m/z* 182 for picric acid and ion at *m/z* 187 for martius yellow), table 4 (Appendix III, p.30). For picric acid, other cleavage associated with the presence of nitro and hydroxyl groups in the molecule, yields the ion at *m/z* 154 which corresponds to the loss of NO_2+CO.

The MS^2 spectrum of the naphtol yellow base peak (ion at *m/z* 313) revealed two major ion products, namely the ion at *m/z* 296 and 233 which corresponds respectively to the losses of the hydroxyl group and the sulfonic group from the $[M-2Na+H]^-$ molecule. In the naphtol yellow, the presence of sulfate groups reduces the stability of the $[M-2Na+H]^-$ molecule [19] yielding the SO_3 fragment ions instead the nitro groups fragments as for picric acid and martius yellow.

3.2.2 Azo dyes (monoazo and disazo)

The azo dyes can be well analyzed using the HPLC gradient with TEA. Structural isomers as metanil yellow (CI 13065) and orange IV (CI 13080) or orange I (CI 14600) and orange II (CI 13080) or fast red E (16045) and ponceau 3RO (CI 16050) were eluted at different retention times, table 4 (Appendix III, p.30). Dyes with sulfonic groups in the *para* position are eluted with a lower retention time than dyes with groups in *meta* or *orto* position. As reported in nitro dyes, the presence of sulfonic groups decreases the retention time of azo dyes. For example, amaranth (CI 16185, rt= 14.25 min) is eluted before than fast red B (16180, rt= 40.10 min), due to the higher content of sulfonic groups. On the other hand, the presence of CH_3 groups and number of aromatic rings contributes to decrease the general polarity of the molecule and increase its retention time as in wool red B (CI 27200) and cotton scarlet (CI 27290) dyes. For example, the wool red B (CI 27200, rt=37,58min) is eluted after cotton scarlet (CI 27290, rt= 25.44 min) due to the presence of two methyl groups in its composition.

In congo red (CI 22120) and cotton scarlet it was observed peak tailing. Nevertheless, these dyes have distinct retention time and UV-Vis spectra.

The majority of the azo dyes have several sulfonic groups in their molecular structure and their negative ion ESI-MS spectrum exhibit typical $[M+ (x+y)H - yNa]^{x-}$ ions. For single sulphonated azo dyes, as metanil yellow, the major fragment ion was $[M-Na]^+$, for di-sulphonated azo dyes, as ponceau 2R (CI 16150), it was detected the $[M-2Na+H]^-$ ion and for tri-sulphonated azo compounds, as amaranth, $[M-3Na+2H]^-$ was the major fragmentation ion observed, table 4 (Appendix III, p.30) [18]. These dyes are usually present in the form of sodium salt and due to its instability are dissociated into $RSO3^-$ and Na^+ ions [19].

The ESI product ion spectrum revealed characteristic fragment ions corresponding to the losses of sulfonic groups from the $[M+(x+y)H - yNa]^{x-}$ fragment ion. It was observed typical neutral losses of SO_3 (for example, ions at *m/z* for 272 for orange IV and ion at *m/z* 355 for ponceau 2R) and SO_2 (ion at *m/z* 288 for orange IV and ion at *m/z* 507 for amido black 10B (CI 20470) groups, table 4 (Appendix III, p.30).

Also, it was detected characteristic fragments from the cleavage of the azo bond (N=N) in the ESI-MS/MS spectrum as the ion at *m/z* 264 in archil red (CI 13355) and the ion at *m/z* 317 in amaranth (CI 16185). Other characteristic fragments, related with the cleavage of the azo bond are the ion at *m/z* 143 in fast red AV (CI 15620) and the ion at *m/z* 302 in orange GG (CI 16230), from the cleavage of the C-N bond.

The MS^n experiments were particular useful in the characterization of molecular isomers which were eluted at similar retention time and with a similar UV-Vis spectrum as with orange II (CI 15510) and crocein orange G (CI 15970), figure 5. The MS full scan of both dyes displayed the ion at *m/z* 327 $[M-Na]^-$ as the base peak [20]. The tandem mass spectrometric (MS/MS) experiments of the $[M-Na]^-$ ion, revealed distinct patterns of fragmentation for each dye. In orange II it was observed the ion at *m/z* 247, corresponding to the loss of SO_3 group and the ion at *m/z* 171 corresponding to the N=N bond cleavage. In crocein orange G it was detected the ion at *m/z* 235, attributed to the loss of N=N and the ion at *m/z* 207, corresponding to the loss of $C_6H_5N_2O$.

Other isomers have the same fragmentation pattern as orange I and II as well as the metanil yellow and orange IV. However the fragment ions products obtained with the MS/MS mode, display different relative abundance, table 4 (Appendix III, p.30). For example, in orange I, the ion at *m/z* 247 (loss of SO_3 group), shows a higher relative abundance than the ion at *m/z* 171 (ion related with the N=N bond cleavage). In orange II (CI 15510), the most abundant peak is the ion at *m/z* 171. Moreover, they have different retention time and UV-Vis spectra, which enables their characterization and differentiation.

Several azo dyes have hydroxyl groups in their composition or carboxylic groups as alizarin yellow GGN (CI 14025), besides SO_3 groups. For these dyes it was observed a loss of 28u and 44u (for instance , the ion at *m/z* 242 for alizarin yellow GGN (CI 14025)) which corresponds to the loss of CO_2 related with a presence of a carboxyl group. In tropaeolin O (CI 14270) , Fast Red AV (CI 15620) and Ponceau 2R (CI 16150) it was observed a loss of 18u, ions at *m/z* 275, 359 and 417, respectively, which corresponds to the loss of a water molecule (H_2O) and is frequently observed in molecules with hydroxyl groups [19]

Some azo dyes are a mixture of several chromophores with different retention time, UV-Vis and mass spectra. The azo flavine (CI 13090) is composed by six red chromophores, which are eluted at different retention times. The main chromophore (1) with a retention time of 30.27min and a λ_{max}= 441nm, is characterized by an ion base peak at *m/z* 397. With the tandem mass spectrometric (MS/MS) experiments it was obtained the ions at *m/z* 333 and 317, which corresponds to the losses of SO_2 and

SO_3, respectively. It was also obtained an ion at *m/z* 156, which indicates the cleavage of C-N bond. These results are in agreement with the molecular structure published in literature [21]. The peak eluted at 35.68min (2) and with a UV-Vis maximum at=401 nm, displays an ion base peak at *m/z* 442. With the negative ESI-MS/MS spectrum it was also detected the losses of SO_2 and SO_3 (ion at *m/z* 378 and ion at *m/z* 362) as in peak (1). The other remaining peaks (3 and 4) are isomers of peak 1. The smaller peaks (5 and 6) were not observed in the ionic chromatogram.

The scarlet N for silk (CI 15635) is composed by two chromophores as described in [8] which are very similar, being eluted at a very close retention time. Both chromophores have a base peak at *m/z* 377. The fragment ions products obtained with the MS/MS mode were *m/z* 221 for the first chromophore and *m/z* 221 and 206 for the second chromophore. The ion at *m/z* 221 corresponds to the azo bond cleavage and the ion at *m/z* 206, corresponds to the C-N cleavage.

The amido naphthol red G (CI 18050) is also composed by two chromophores which are eluted at a close retention time, but with a different UV-Vis maximum, table 4 (Appendix III, p.30). The first peak has a base peak at *m/z* at 422, which corresponds to the azo dye fast acid magenta (CI 17200). The negative ESI-MS/MS spectrum confirmed the presence of the characteristic fragment ions at *m/z* at 358 from the losses of SO_2, from the $[M-2Na+H]^-$ fast acid magenta dye. The second peak with a base peak at *m/z* at 464, corresponds to the $[M-2Na+H]^-$ molecular structure published in literature for amido naphtol red G (CI 18050) [21]. These results suggest that the amido naphthol red G (CI 18050) is a mixture of two chromophores, being one of them, the fast acid magenta dye (CI 17200) compound reported in the literature [21].

The azo fuchsine 6B (CI 18055) is also composed by three chromophores eluted at different retention times, as described in [8]. The chromophores 1, 2 and 3 are characterized by a base peak at *m/z* 437, 479 and 521 respectively. The ion of chromophore 3 and the fragmentation pattern observed (table 4, Appendix III, p.30) is in agreement with the molecular structure published in the literature [21]. The tandem mass spectrometric (MS/MS) experiments for the chromophores 2 and 1, suggests that these chromophores are related with chromophore 3, with a less number of $COCH_3$ groups in their structure. This is in agreement with the elution retention time obtained for the three chromophores, where chromophore 3, with a higher number of $COCH_3$ groups, is eluted after chromophore 2 and 1.

Chrysoidine (CI 11270) was the only azo dye that was detected in the positive mode, due to the absence of sulphates groups and presence of amine groups. The positive ion ESI-MS spectrum of chrysoidine (CI 11270) exhibit the $[M+H]^+$ ion as the base peak. The ES-MS/MS spectrum revealed two major ion products, the ion at *m/z* 196 and 121 which corresponds respectively to the losses of ammonia and the N-N bond cleavage, table 4 (Appendix III, p.30).

The vesuvine 2B (CI 21000), contrarily to what expected, was not detected in ES negative or positive mode. Nevertheless, this dye has a different UV-Vis spectrum and retention time from the other dyes analyzed, being possible to correctly identify and distinguished it in a historical textile sample with the information obtained from the DAD detector.

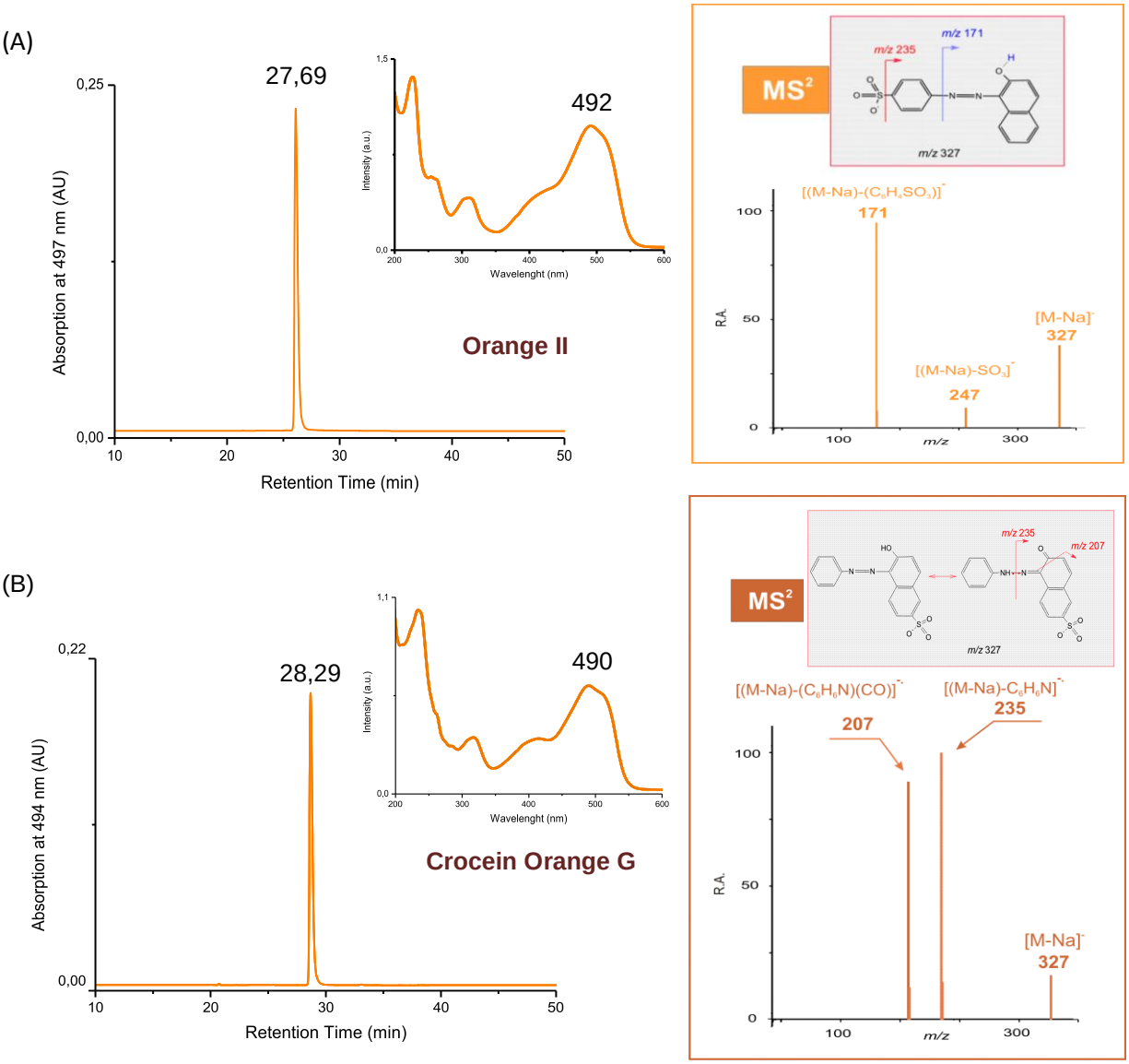

Figure 5- HPLC-DAD chromatogram and MS^2 spectrum of the $[M\text{-}Na]^-$ ion *m/z* in negative mode, for Orange II (CI 15510) (A) and Crocein Orange G (CI 15970) (B).

3.2.3 Methane dyes (triaryl and diphenyl)

The triarylmethane dyes and diphenylmethane dyes are usually composed by several chromophores as fuchsine (CI 42510), methyl violet (CI 42535), victoria blue R (CI 44040), etc, table 4 (Appendix III, p.30). These dyes were efficiently separated, being possible to identify the several compounds in their composition. Chromophores with a higher number of methyl groups as methyl violet (CI 42535), crystal violet (CI 42555), victoria blue R (CI 44040) and victoria blue B (CI 44045) as expected, revealed a higher affinity with MeOH phase and were eluted at later retention times.

Most of these substituted trialylmethane salts were analyzed in positive ESI mode due to their structure with methyl groups, showing characteristic fragmentation patterns.

The MWs, ions observed in the LC-MS spectra and product ions obtained in the LC-MS/MS spectra are listed in Table 4 (Appendix III, p.30). The positive ion spectrum of methane dyes exhibit the molecular cation, $[M]^+$ as the base peak. The MS/MS spectrum usually exhibit the loss of the hydrocarbons as well as the loss of one aromatic ring (-78u) from the $[M]^+$ cation. For example, the malachite green gives a

peak at *m/z* 329 corresponding to the intact cation, $[M]^+$. The product ion spectrum obtain from $[M]^+$ shows peaks at *m/z* 313 and 314 corresponding to a CH_4 and $^{\bullet}CH_3$ losses, respectively; the peak at *m/z* 286 results from a NC_2H_5 fragmentation. In malachite green it was observed the fragmentation of the aromatic ring (ion at *m/z* 251) as well as the fragmentation of the aromatic ring with $N(CH_3)_2$ (ion at *m/z* 208) in agreement with the literature [21].

Fuchsine and Methyl Violet are composed by several chromophores eluted at different retention times. The ESI spectrum exhibit the $[M]^+$ ion for each chromophore (the ions at *m/z* 288 (chromophore 1), 302 (2), 316 (3) and 330 (4) for fuchsine and the ions at *m/z* 330 (1), 344 (2), 358 (3) and 372 (4) for methyl violet, as reported in literature [21]. Typical fragment ions related with the loss of methyl groups were also obtained (ion at *m/z* 287 for chromophore 1 in fuchsine and the ion at *m/z* 329 for chromophore 1 in methyl violet) in MS^2 mode. It was also observed the loss of one aromatic ring with an amino group for fuchsine (ion at *m/z* 223 in chromophore 3) or the loss of a $NHCH_3$ group for methyl violet (ion at *m/z* 251 in chromophore 1) . The chromophore (4), which corresponds to the fully methylated form of methyl violet has the same molecular structure of crystal violet, as described in the literature [8].

Methyl blue (CI 42780) was analyzed in negative mode due to the presence of sulfonic groups. The negative ESI-MS spectrum exhibit the characteristic $[M-2Na+H]^-$ parent ion for this dye.

The light green SF bluish (CI 42075) revealed a non-expect ion base peak (*m/z*=373), according with the structure published in the literature. Further NMR studies are necessary to confirm and elucidate the structure of this dye.

Patent blue V (CI 42052) had no signal with LC-MS analysis in positive or negative mode. Nevertheless, when analyzed by direct infusion it was found a peak at *m/z* 667 and 669, in the negative and positive spectrum, respectively. This peak is in agreement with the molecular structure of Patent Nlue V. This triarylmethane dye has a retention time and UV-Vis spectrum maximum related with brilliant green (CI 42040). Nevertheless it can be distinguished from the previous one, since the brilliant green exhibit the characteristic fragment ion at *m/z* 385.

3.2.4 Xanthene, azine and thiazine dyes

Xanthene dyes presents carboxylic acids groups in their composition, and as a result they usually have lower retention times than triarylmethane dyes. Dyes with similar structures, as eosine A (CI 45380) and erythrosine (CI 45430) have similar UV-Vis spectrum and they were eluted at similar retention time, table 4 (Appendix III, p.30). Nevertheless, they were distinguished easily due to their mass spectrum once eosine has bromine groups and erythrosine iodide groups in their structure.

The azine and thiazine dyes, are complex dyes, with several chromophores in their composition which could be very well separated, table 4 (Appendix III, p.30).

The positive ion ESI-MS spectrum for azine and thiazine dyes exhibit the characteristic $[M]^+$ parent ion. In xanthene dyes it was found $[M-XNa+YH]^{+/-}$ as the base peak (Uranine (CI 45350), Eosine and Erythrosine) or $[M-HCl]^+$ (Rhodamine 6G (CI 45160).

The tandem mass spectrometric (MS/MS) experiments for the xanthenes and thiazine dyes exhibit the effect of ethyl groups by the typical losses of the C_2H_5 (ion at *m/z* 386 for rhodamine 6G and *m/z* 414 for rhodamine B (CI 45170) or NC_2H_5 (the ion at *m/z* 241 in methylene blue (CI 52015) group from the $[M]^+$ cation. Other characteristic cleavage observed in xanthenes group is the loss of CO (the ion at *m/z* 415 for rhodamine 6G and the ion at *m/z* 305 for uranine A) and CO_2 (ion at *m/z* 399 for rhodamine B) related with the presence of carboxylic groups in their composition.

In uranine A, a loss of 18u was observed corresponding to the loss of H_2O, as described previously. Other characteristic fragmentation related with the presence of hydroxyl groups [11] are the ion products at *m/z* 287 and 272, which corresponds to the losses of H_2O+CO and H_2O+CO_2, respectively.

Mauve dye (CI 50245) is a complex mixture of several chromophores, all containing the 7-amino-5-phenyl-3-(phenylamino)phenazin-5-ium core with different number of methyl groups in their molecular structure [22]. In the mauve dye standard from the Science Museum, figure 5 it was detected the presence of 4 main chromophores described in the literature [22]: 1- mauveine C25a and C25b ($[M]^+$ *m/z* 377), 2- mauveine A ($[M]^+$ *m/z* 391), 3 - mauveine B ($[M]^+$ *m/z* 405) and 4-mauveine C ($[M]^+$ *m/z* 419).
In the mauve dye from the Schweppe collection it was possible to identify the presence of pseudo-mauveine as the majour chromophore, as reported in the mauve salt from the Schunk collection [22].
Figure 6 shows the HPLC-ESI/MS total ion chromatogram of mauve dye from the Science Museum and the inserts show the HPLC-ESI/MS^2 ion chromatogram and the mass spectrum for each chromophore. Several characteristic fragment ions related with amine groups were observed. For example it were detected ions at *m/z* 360 for chromophore 2 (-CH_3+NH_2), m/z 361 (-NH_2), 403 for chromophore 4 (-NH2) and *m/z* 284 related with the N-C bond cleavage for chromophore 1 (-C_6H_7N).

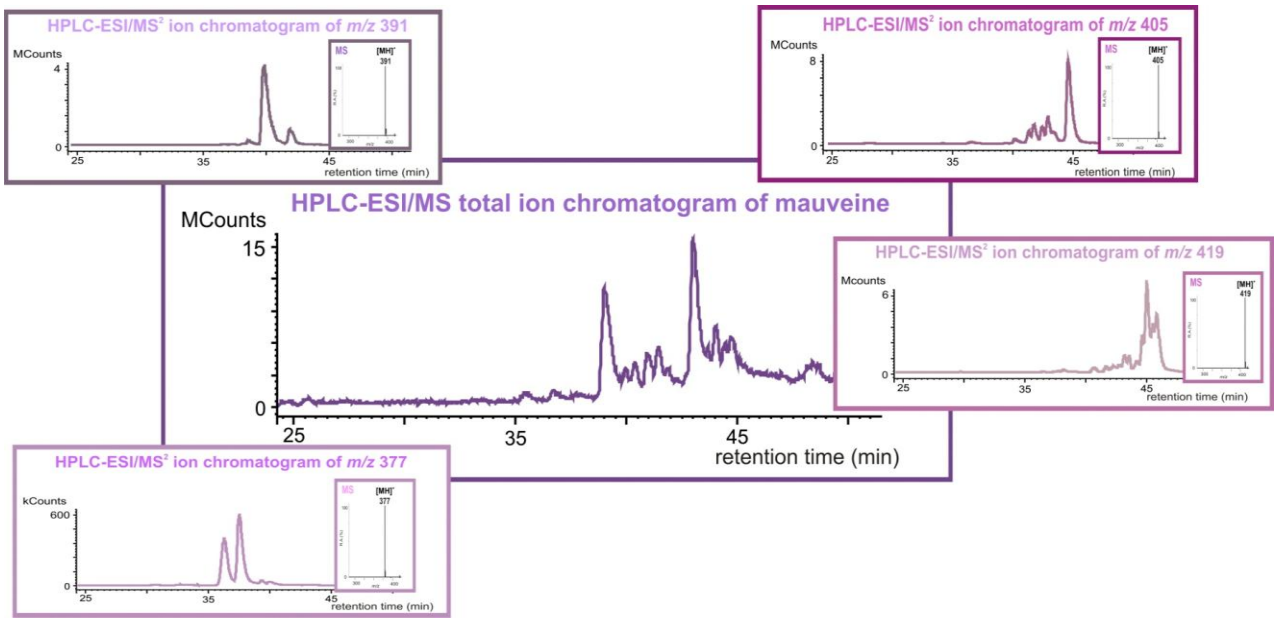

Figure 6- HPLC-ESI/MS total ion chromatogram of mauve dye from the Science Museum. HPLC-ESI/MS^2 ion chromatogram and the mass spectrum of each mauveine chromophore.

Eosine A and erythrosine were analyzed in negative mode due to the presence of sodium in their molecular structure. The negative ESI-MS spectrum exhibit the $[M-2Na+H]^-$ ion as a base peak for these dyes. It was also detected the characteristic fragmentation related with carboxylic groups, table 4. For eosine A, the $[M-2Na+H]^-$ at *m/z* 647 presents the expected isotopic distribution for a compounds with four bromine atoms in its structure. The MS/MS experiments for the $[M-2Na+H]^-$ molecules of these dyes, exhibits the typical loss of CO_2 (ion at *m/z* 603 for eosine A and the ion at *m/z* 791 for erythrosine). Both dyes exhibit distinct fragmentation patterns, where in Eosine it was detected fragmentations related with the presence of Br atom as the $Br+CO_2H$ and Br_2+CO_2H fragmentation (ion at *m/z* 523 and 443 respectively). In Erythrosine it was detected typical losses related with the presence of iodide e atom as the $I+CO_2H$ and I_2+CO_2H fragmentation (ion at *m/z* 663 and 537 respectively).

The positive ESI/MS spectrum for safranine T (CI 50240) and nigrosine (CI 50420), from azine dyes, were not conclusive as the molecular ions obtained did not match with the molecular weight reported in the literature. It is possible that, as with the mauve dye, their molecular structure is not fully elucidated. Both dyes (nigrosine and safranine) are also obtained from coal-tar derivatives. Depending of the starting materials, their synthesis might produce different chromophores [22]. Further NMR studies are necessary to confirm and elucidate the structure of these dyes.

3.2.6 **Quinoline, Anthraquinone, Flavonoid, aminokectone/lactone and indigoid dyes**

The antraquinone and aminokectone/lactohne dyes have a considerable number of OH groups in their composition. Murexide (CI 56085) is almost unretained in the system due to its high polarity, however good results were obtained for Alizarin (CI 58000) and Alizarin Red S (CI 58005), table 4 (Appendix III, p.30). The natural antraquinone dye, cochineal, was also very well resolved with this system.

Fragmentation varies according to the presence of different groups in these dyes structure. Alizarin red s tends to lose Na and SO_3 due to the presence of sulfonic groups and Murexide lose NCHO fragments due to the presence of OH groups, table 5.

The flavonoid based dyes (weld) were very well resolved with the TEA system and the mass spectrums obtained in full scan and MS/MS mode were in agreement with the literature [16].

Although indigo and indigo carmine have a similar UV-Vis spectrum, it is possible to distinguish them, by their retention time and mass spectrum. As expected, indigo carmine is eluted before indigo due to the presence of two sulfonic groups, table 4. On Ms^n analysis, Na and SO_3 are the first losses for indigo carmine.

The negative ESI-MS spectrum exhibit the $[M-H]^-$ ion or the $[M-Na]^-$ for dyes with sulfonic groups in their molecular structure, as alizarin red S and indigo carmine. The presence of carbonyl groups lead to the loss of CO, shortening an aromatic ring by one carbon atom [11] (ion at *m/z* 221 for alizarin and *m/z* 415 for indigo carmine). The effect of sulfonic group is given by the losses of SO_2 and SO_3 (respectively, the ion at *m/z* 255 and 239 for alizarin red S and the ion at *m/z* 379 and 363 for indigo carmine). In murexide ES-MS/MS spectrum it were detected losses of 43u and 86u which corresponds to the NCHO and $(NCHO)_2$ fragmentations.

3.3 **Persian Carpet 20th century**

The bright and vivid red, blue, green and yellow colours from Persian Carpet T107 (Appendix IV, p.45) were all obtained with synthetic dyes. The HPLC-DAD-MS^n analysis, excluded the utilization of the common natural dyes used supposedly by the Armenian weaver.

In the HPLC-DAD chromatogram of the red textile samples, figure 7 it was detected the presence of four principal chromophores eluted at 1 – 23.10 min, with UV-Vis maximum at 528 nm, 2 – 39.00 min, with UV-Vis maximum at 514 nm, 3- 40.13 min, with UV-Vis maximum at 512 nm and 4- 41.80 min, with UV-Vis maximum at 548 nm. The negative ESI-MS spectrum revealed the presence of ion products at *m/z* 511 for peak 1 which may corresponds to cotton scarlet (CI 27290). For peak 2, 3 and 4 it was detected the ion at *m/z* 377, figure 7. The peaks 2 and 3 have identical *m/z* values, retention time and UV-Vis spectrum to the scarlet n for silk (CI 15635) standard in the HPLC-DAD-MSn library. The peak 4 was not identified. Based on the mass results, retention time and UV-Vis spectrum, this compound is also probably from the same chemical family of peak 2 and 3, the azo dyes family. It is necessary to perform tandem mass spectrometric (MS/MS) experiments in order to identify the correct molecular structure of peak 4 as well as to confirm the other peaks.

The HPLC-DAD chromatograms of the yellow and beige colours, figure 8 were identical, displaying both seven main chromophores, namely: 1 with a retention time of 28.35 min and λmax= 437 nm; 2- 29.27 min with 441 nm; 3- 32.77 min with 403 nm; 4- 33.25 min with 415 nm; 5- 34.50 min with 407 nm; 6- 35.28 min with 447 nm and 7- 38.47 min with 403 nm. The yellow colours were more concentrated than beige colours, circa six times.

The negative ESI-MS spectrum for a yellow sample revealed the ion at *m/z* 352, which corresponds to orange IV (CI 13080) and the ions at *m/z* 397 and 442, which correspond to azo flavine 3R (CI 13090), as shown in figure 9. Their retention times and UV-VIS spectrums also matches with the synthetic dyes standards, from the HPLC-DAD-MS^n database, table 4 (Appendix III, p.30).

The HPLC-DAD chromatograms of the blue samples, figure 10 excluded the utilization of the common natural indigo dye and pointed out the utilization of synthetic blue dyes. The chromatogram acquired at 634 nm revealed the presence of six blue chromophores, namely 1 with retention time at 19.08 min and λmax= 593 nm, 2 with retention time at 19.33 min and λmax= 605 nm, 3 with retention time at 20.28 min and λmax= 578 nm, 4 with retention time at 20.75 min and λmax= 606 nm, 5 with retention time at 22.20 min and λmax= 620 nm and 6 with retention time at 24.05 min and λmax= 634 nm. These UV-Vis spectrums are similar to the blues from the triarylmethane family. The positive ESI-MS spectrum of a blue sample had a very low signal and it is necessary to repeat the analysis with a sample with circa 0.5 mg.

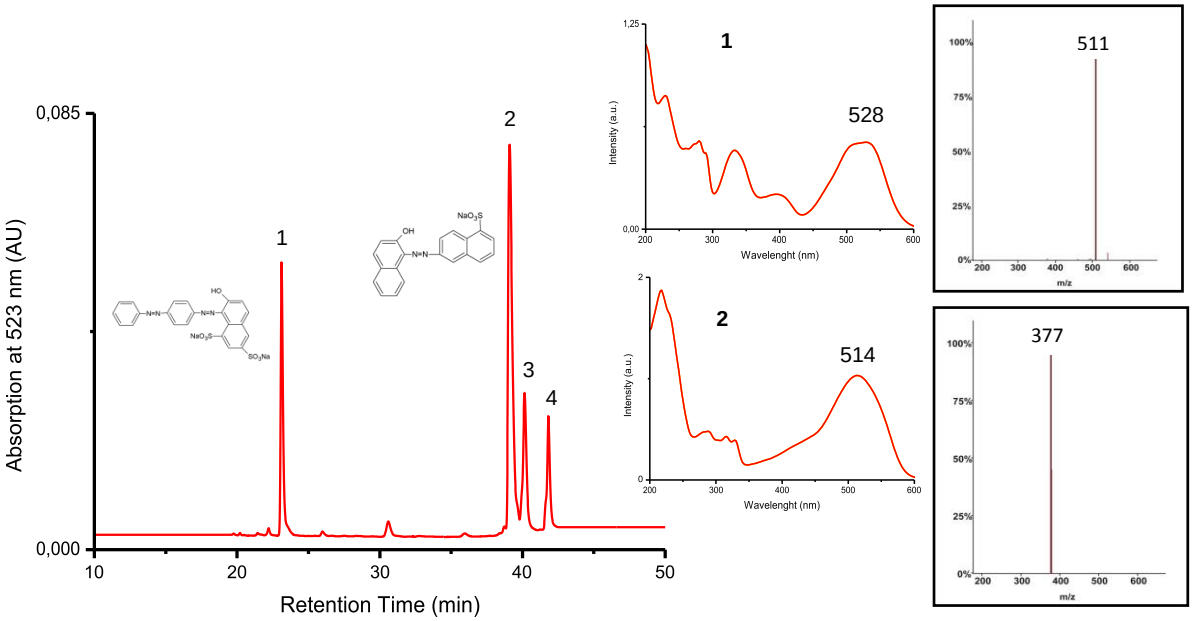

Figure 7– HPLC chromatogram, UV-Vis spectra and negative-ion ESI mass spectra of the red colour fiber from Persian Carpet T107.

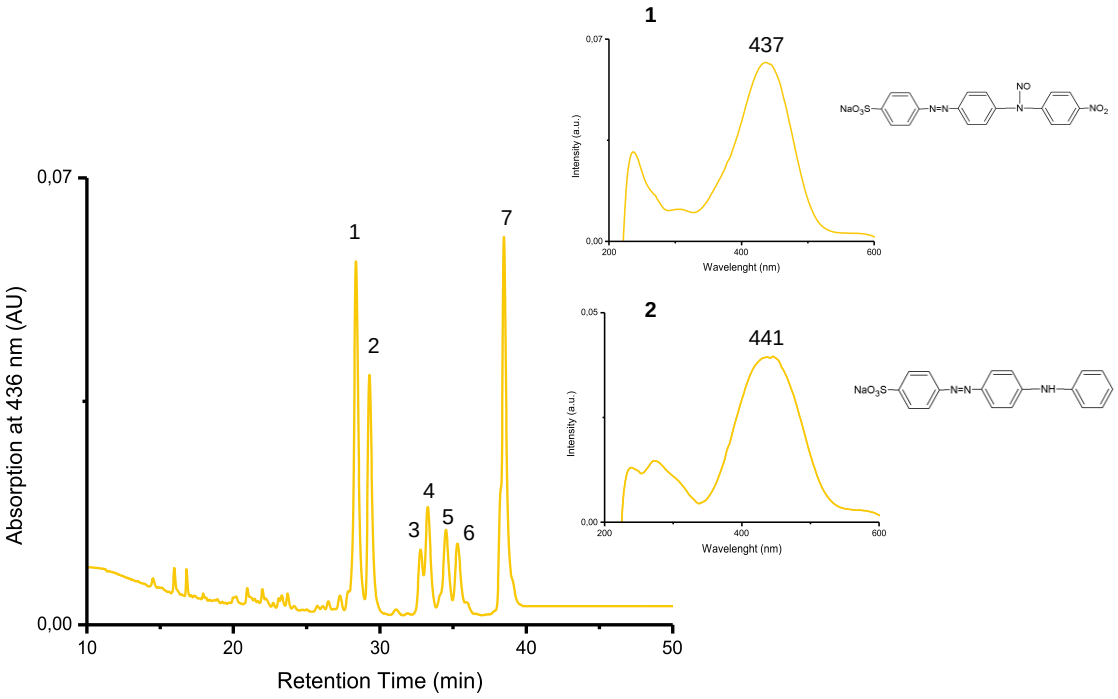

Figure 8- HPLC chromatogram and UV-Vis spectra of the yellow colour from Persian Carpet T107. Beige and green colours presented identical chromatogram and UV-Vis spectra.

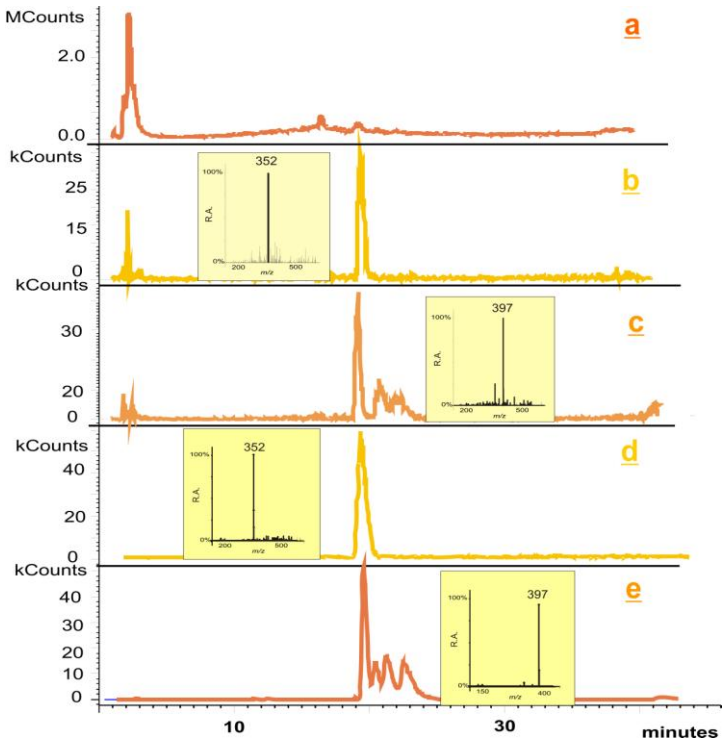

Figure 9 – (a) Total ion chromatogram obtained in the ESI negative mode for an yellow fiber extract from Persian Carpet T107; (b) extract ion chromatogram and mass spectrum of the *m/z* 352 ion; (c) extract ion chromatogram and mass spectrum of the *m/z* 397 ions; (d) ESI-MS/MS ion chromatogram and mass spectrum for ion *m/z* 352 from orange IV dye; (e) ESI-MS/MS ion chromatogram and mass spectrum for ion *m/z* 397 from azo flavine 3R dye.

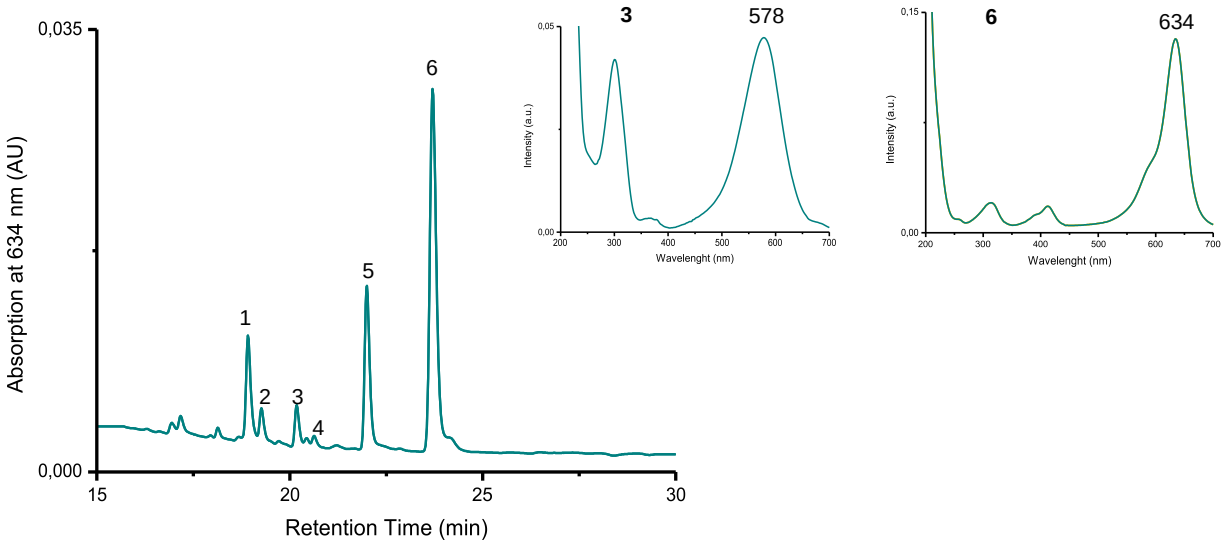

Figure 10 - HPLC chromatogram and UV-Vis spectrums of the blue colour from Persian Carpet T107. Green colour presented identical chromatogram and UV-Vis spectra.

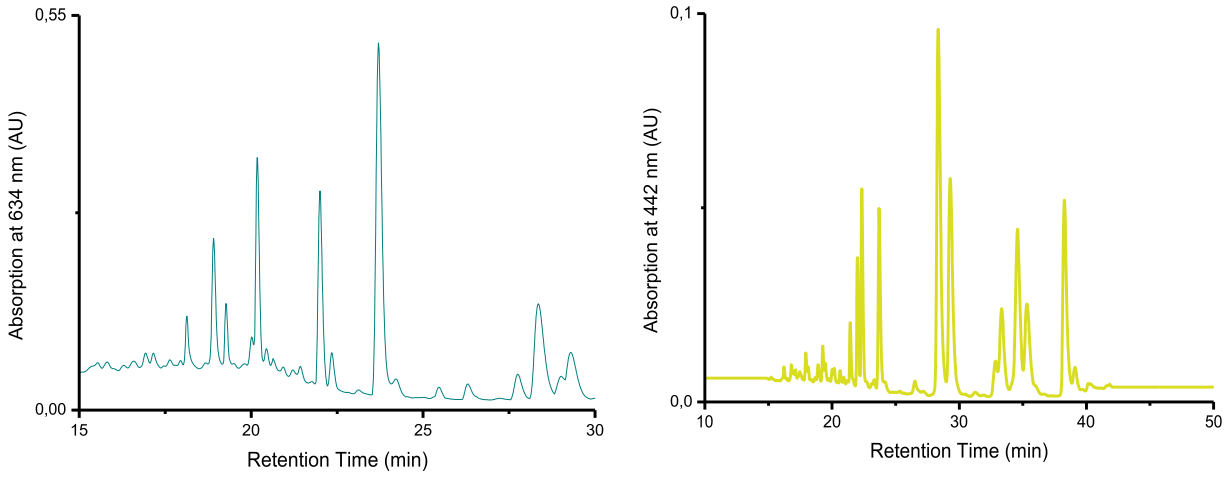

Figure 11 – HPLC chromatograms (at 634 and 442 nm) of the green colour from Persian Carpet T107.

It was possible to conclude that the red colours were obtained with a mixture of at least two synthetic dyes (scarlet n for silk (CI 15635) and cotton scarlet (CI 27290)); the yellow and beige colours were obtained both with a mixture of two synthetic dyes (azo flavine 3R (CI 13090) and orange IV (CI 13080)); the blue colours were obtained with synthetic dyes and the green colours, figure 11, were obtained with a mixture of azo flavine 3R, orange IV (previously identified in yellow and beige colours) and the blue synthetic dyes. The blue synthetic dyes present in the blue and green colours was not identified, due to the low amount of sample collected, table 3 (Appendix II, p.27-29).

4. Conclusion

The utilization of triethylamine (TEA) solvent in the mobile phase allowed the separation of a considerable range of synthetic dyes with different structures, as well as complex mixtures of dyestuffs and subsequent analysis with mass spectrometry. They were successfully identified and characterized by their retention time, UV-Vis spectrum and fragmentation patterns.

The combination of both DAD and MS detectors was fundamental for their characterization, as there are hundreds of early synthetic dyes and some of them can display similar structures or UV-Vis spectra. Also much more information about the dye structure can be obtained, namely with mass spectrometry, which is particularly important in unidentified samples.

The successful application of HPLC-DAD-Ms^n technique for the identification of the early synthetic dyes depend critically on the creation of specific synthetic dyes libraries as the one performed in this work. Indeed, the chemical synthetic dyes classes can display a characteristic pattern which can be characterized and distinguished with the HPLC-DAD-Ms^n system. The LC-ESI-MS^n fragmentation ions of the 62 dyes revealed specific fragmentation related with the presence of specific functional groups (carboxylic, hydroxyl, sulfonic, amino groups, just to name a few) present in their molecular structure, which in a first approach can contribute to identify the chemical family in a synthetic dye. For instance, in dyes containing sulfonic groups, the negative ion ESI-MS spectrum exhibit typical [M+ (x+y)H -

yNa]$^{x-}$ ions and typical neutral losses of SO_3 or SO_2 from the [M+(x+y)H - yNa]$^{x-}$ fragment ion, but when the sulfonic groups are absent as in carboxylic acids, then the neutral loss of CO_2 (*m/z* 44) is the first typical fragmentation path.

As each molecule has specific fragmentation product ions or retention times or UV-Vis spectrum, unidentified dyes can be well identified by comparison with the database HPLC-DAD-Ms^n library.

This was the case of the carpet T107 dyed-textile samples, where it was identified the presence of azo flavine 3R (CI 13090) and orange IV (CI 13080) for yellow, beige and green colours and cotton scarlet (CI 27290) and scarlet n for silk (15635) for red colour. All the dyes identified are from the azo family with sufonic groups in their composition. The blue colour of the carpet T107 was not present in the early synthetic dyes Schweppe database. However with the information obtained from the HPLC-DAD and comparison with the HPLC-DAD-Ms^n library created, its chemical family could be suggested (triarylmethane dyes). Further tandem mass spectrometric (MS/MS) experiments are necessary to correctly identify the structure of this dye.

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Appendixes

Appendix I - Schweppe´s Collection

1.1 Early synthetic dyes

For more than 4000 years, the dyes used in works of art were obtained from natural sources, namely insects as cochineal, kermes, or lac (reds) or plants as indigo (blue) and weld (yellow).

It was only in 1771, that Peter Woulfe discovered picric acid, made from indigo and nitric acid [1]. In 1840s, it started to be commercialized as a dyestuff; however it never became to be largely industrialized due to its explosive nature.

It was Henry Perkin that discovered in 1856 the first synthetic dye commercialized in a large scale. This purple dye was initially named Aniline Purple or Tyrian Purple as an analogy to the purple natural dye. This dye became to be known by the name of Mauveine, adopted in association with the pale-violet mallow flower well known in France. This became the favorite color of Empress Eugenia and Queen Victoria, spreading all over France and England [2]. Very soon, others synthetic dyes from coal-tar chemicals, as magenta, methyl violet, Hofmann's violet and aniline black [1], were discovered and made available in a large scale, launching the synthetic industry in Europe (UK, France, Germany and Switzerland) and later in USA. By 1890, 90% of all dyestuffs were of synthetic origin [3]. In 1868, Graebe and Liebermann made the first artificial synthesis of a natural organic dye. It was the synthesis of alizarin [1]. Following these new discoveries, was the development of the organic chemistry and a great step was the establishment, in 1865, of Kekulé's benzene theory [1].

Beside the synthesis of dyes equivalent to natural dyes, as indigo (synthesized in 1897) new colours, as the azo dyes, unknown in natural dyes, were manufactured with a particular chromophore or colour-producing group [1].

The chemist Helmut Schweppe was interested on these dyes that were largely used between 1850 and 1900. On his work about textiles dyeing Reference Collection DYE 16850-16915, "Important Early Synthetic Dyes" and dyed fibers "Colourings of Early Synthetic Dyes", he developed simple and practical methods of natural and synthetic dyes differentiation without resorting to sophisticated and expensive analytical methods. There are many museums without sophisticated equipment, so these tests are of great importance because it gives practical information for the identification of 66 early synthetic dyes in works of art, table 1. These practical tests include extraction methods that enable the identification of the family class, besides tests for identification of synthetic dyes through UV fluorescence, among others. All these preliminary tests are compiled in a table where are the results related to the reaction of each synthetic dye in each test, table 1. The use of Thin Layer Chromatography (TLC) is also referred, because in many cases, there is not sufficient amount available for the preliminary tests and spot reactions and this is the main disadvantage of his practical methods. It is needed some significant quantity of sample to make these tests and the results may not be conclusive.

Table 1: Sulfuric acid reaction of some dye groups [3]

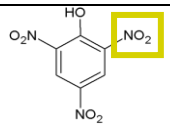
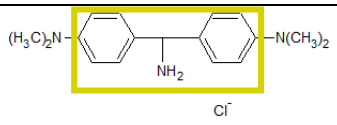
Dye group	Solution color in 98% sulfuric acid
Nitro dyes (Dyes 1-3)*	Yellow to orange
Xanthene Dyes (Dyes 50-54)*	Yellow to red
Triphenylmethane dyes, basic, acid (Dyes 39-49)*	Yellow, orange, red-brown
Azine dyes (Dyes 56-58)*	Green, black
Thiazine dyes (Dye 59)*	Green
Azo dyes (Dyes 4-38)*	Yellow to green
Anthraquinone dyes (Dyes 61-64)*	Yellow to green

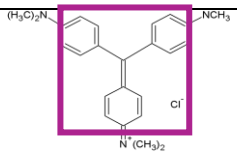
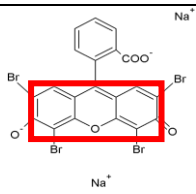
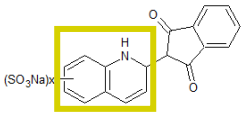
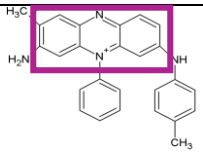
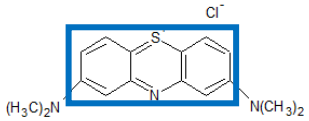
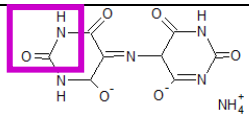
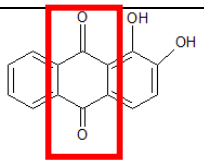
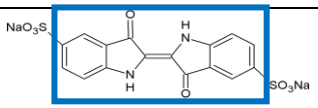
- Numbers from the list of 66 “Important early synthetic dyes”

1.2 Early synthetic dye classification

The 62 early synthetic dyes from the Helmut Schweppe collection can be classified according to their chemical structure, being possible to find 11 chemical families (azo, triarylmethane, xanthene, anthraquinone, azine, nitro, diphenylmethane, quinoline, thiazine, aminoketone/lactone and indigoid) as it is shown in table 1. Besides, each dye has a specific color (red, yellow, blue, orange, violet, brown, black or green) and method of application (acid, basic, direct or mordant) as can be seen in table 2.

Table 2: Example of dyes structures for the eleven chemical families.

Nitro (bond with nitro groups)	 Picric Acid (CI 10305, Acid **Yellow**)
Azo (bond with sulfonic groups)	 Azo Flavine 3R (CI 13090, Acid **Orange** 1)
Diphenylmethane (bond with amine groups)	 Auramine O (CI 41000, Basic **Yellow** 2)

Triarylmethane (bond with amine groups)	 Crystal Violet (CI 42555, Basic **Violet** 3)
Xanthene (bond with amine groups)	 Eosine (CI 45380, Acid **Red** 87)
Quinoline (bond with sulfonic groups)	 Quinoline (CI 47005, Acid **Yellow** 3)
Azine (bond with amine groups)	 Mauveine A (CI 50245, Basic Dye)
Thiazine (bond with amine groups)	 Methylene Blue (CI 52015, Basic **Blue** 9)
Aminoketone/Lactone (bond with OH groups)	 Murexide (CI 56085, Mordant Dye)
Anthraquinone (bond with OH groups)	 Alizarin (CI 58000, Mordant **Red** 11)
Indigoid	 Indigo Carmine (CI 73015, Acid **Blue** 74)

Appendix II – Experimental section

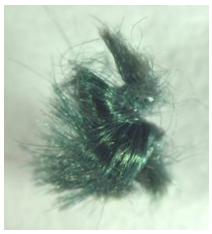
2.1 Samples collected from Persian Carpet T107

9 samples were collected from the Persian carpet T107, figure 1: 1 blue, 1 beige, 2 yellow, 1 green and 4 red. Each sample weighted between 0.3-2 mg. Five different colours were selected for HPLC-DAD-MS^n analysis: blue, yellow, beige, green and red. For the HPLC-DAD analysis it was used a sample with 0.2 mg and for LC-ESI-MS it was needed between 0.3-0.6 mg.

Figure 1 – General photographic picture of the Persian Carpet T107 with sampling location:

1– green, 2- red, 3- blue, 4- yellow, 5- beige.

Table 3 – Pictures of the fibers and sampling location

Location	Sampling	Microscopical Picture	Total weight (mg)
1		 1,6x amplification	0.7 *(sample not fully used)*
2		 2,0x amplification	2.0 *(sample not fully used)*
3		 0,71x amplification	0.8 *(sample not fully used)*
4		 2,0x amplification	1.9 *(sample not fully used)*

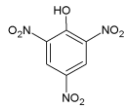
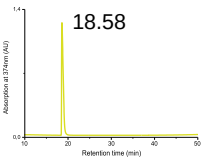
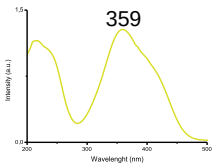
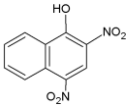
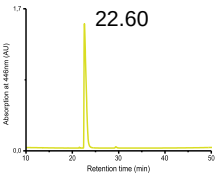
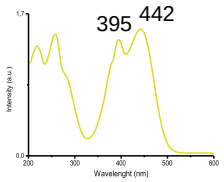
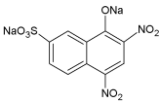
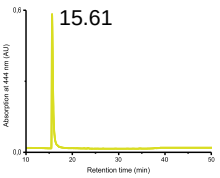
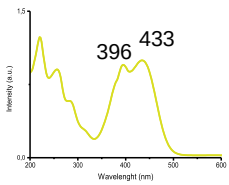
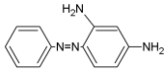
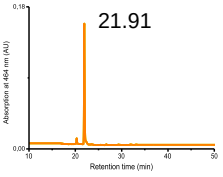
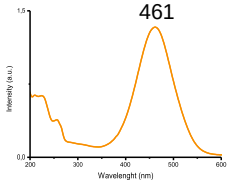
5		 2,5x amplification	1,1

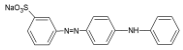
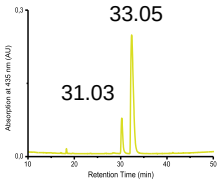
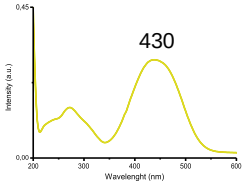
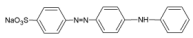
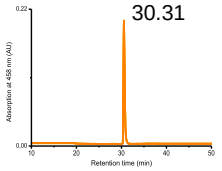
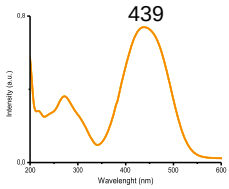
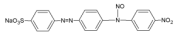
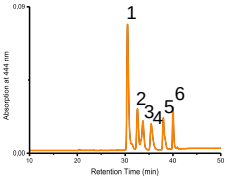
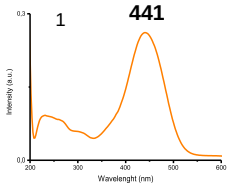
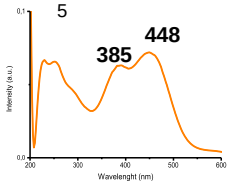
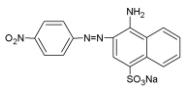
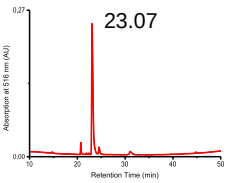
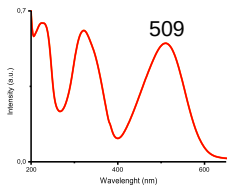
Appendix III - Smithsonian´s Helmut Schweppe Collection HPLC-DAD-MS^n Database

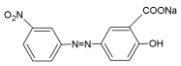
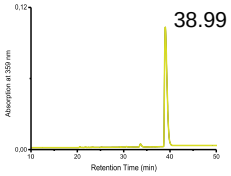
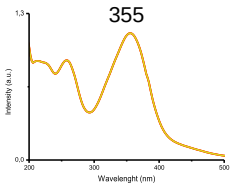
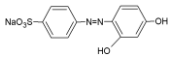
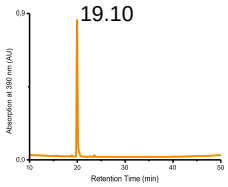
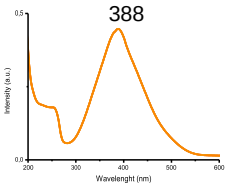
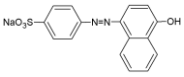
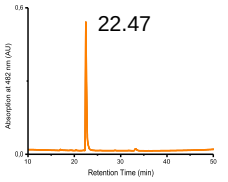
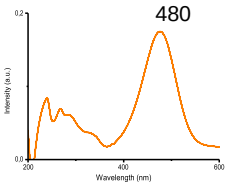
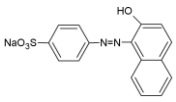
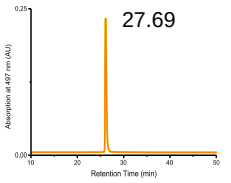
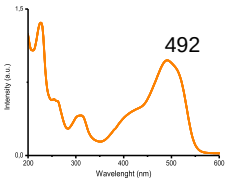
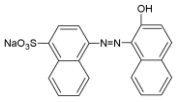
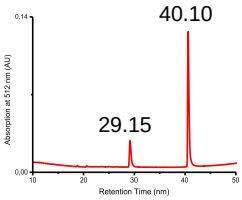
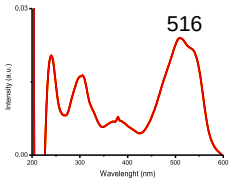
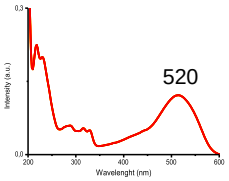
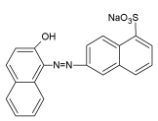
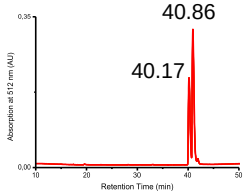
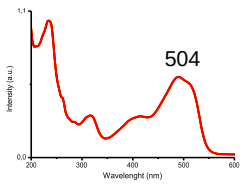
The analysis of 62 early synthetic dyes from the Smithsonian´s Helmut Schweppe Collection with High Performance Liquid Chromatography – Diode Array Detection – Mass Spectrometry (HPLC-DAD-MS^n) was performed. Several chromatographic and mass parameters were optimized to characterize the 62 dyes from the 11 different chemical families. With the results obtained it was possible to perform a database (table 2) which will be helpful for analysis of dyed objects from the second half of 19th century.

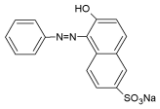
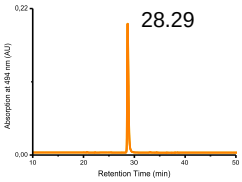
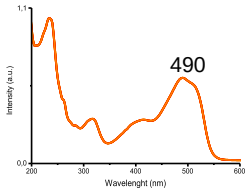
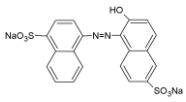
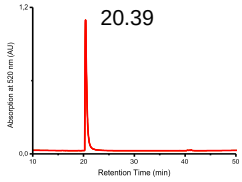
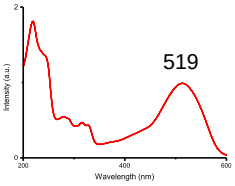
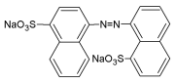
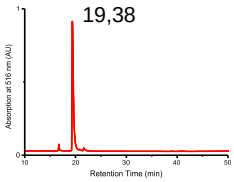
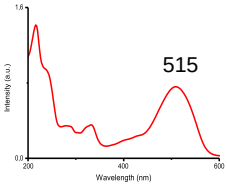
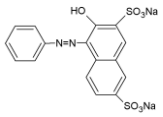
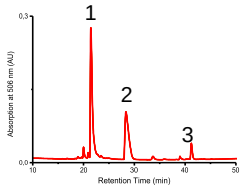
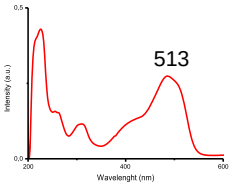
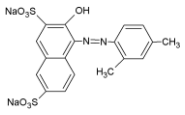
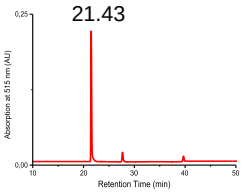
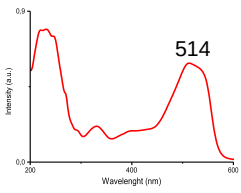
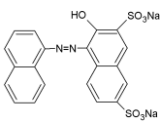
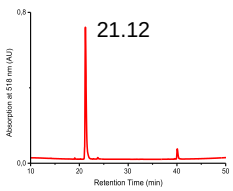
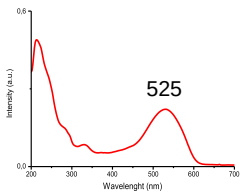
Several natural dyes were analyzed by HPLC-DAD, using the same elution method as for early synthetic dyes (table 4).

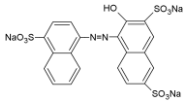
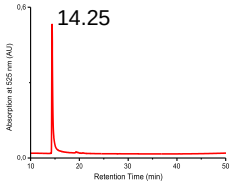
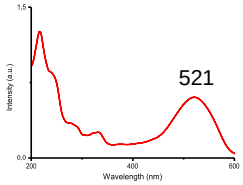
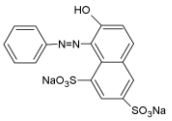
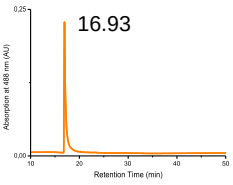
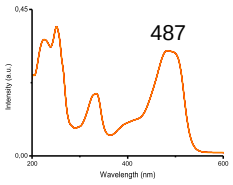
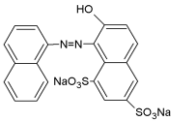
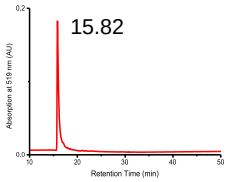
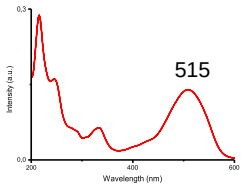
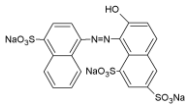
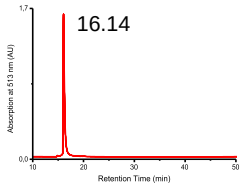
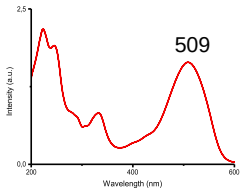
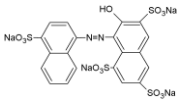
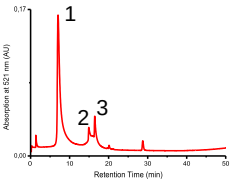
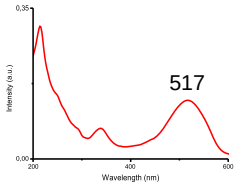
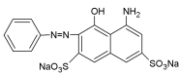
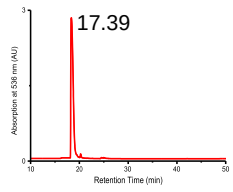
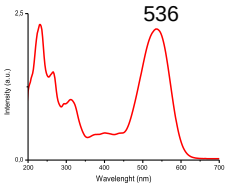
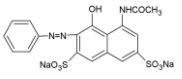
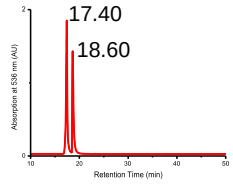
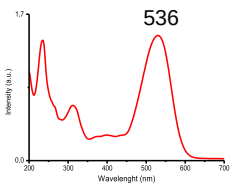
Table 4: Early synthetic dyes database

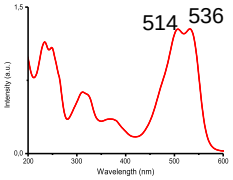
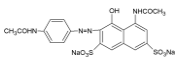
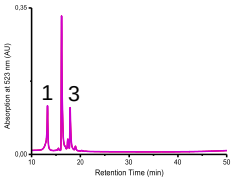
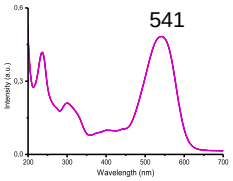
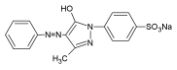
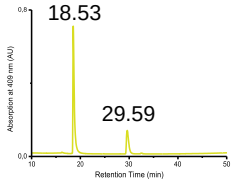
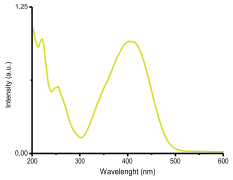
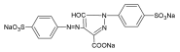
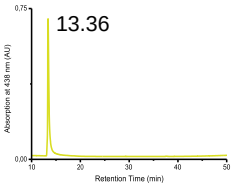
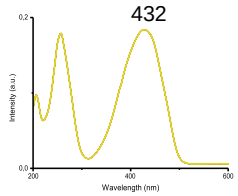
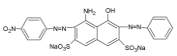
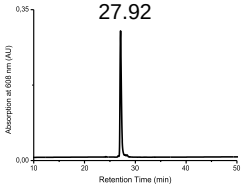
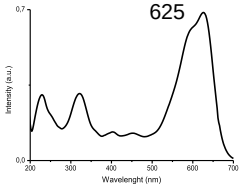
Compound	Structure	Chromatogram (rt, min)	UV-Vis Spectrum (λmax, nm)	MW (g/mol)	MSn fragments *m/z* (%relative abundance) (neutral fragment loss, possible species)
Nitro					
CI 10305 **Picric Acid** Acid Yellow				229.10	MS **m/z 228 [M-H]$^-$** MS^2 **228** → 198 (100)(-30,NO) → 182 (25)(-46, NO_2) → 154 (7) (-74, NO_2+CO)
CI 13015 **Martius Yellow** Acid Yellow 24				234.17	MS ***m/z* 233 [M-H]$^-$** MS^2 **233**→ 203 (25) (-30,NO) → 187 (4) (-46,NO_2)
CI 13016 **Naphthol Yellow S** Acid Yellow 1				358.19	MS ***m/z* 313 [M-2Na+H]$^-$** MS^2 **313**→ 296 (16)(-17,OH) → 233 (100)(-80, SO_3)
Monoazo					
CI 11270 **Chrysoidine** Basic Orange 2				212.25	MS ***m/z* 213 [M+H]$^+$** MS^2 **213**→ 196 (79) (-17, NH_3) → 121 (100) (-92, C_6H_6N)

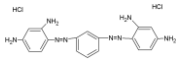
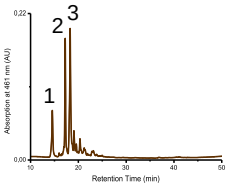
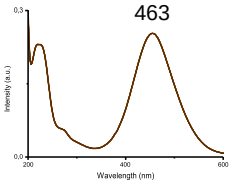
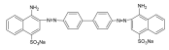
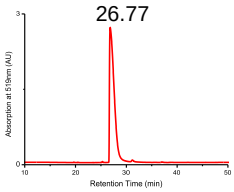
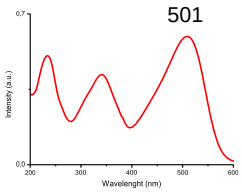
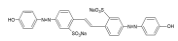
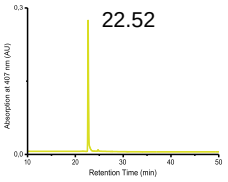
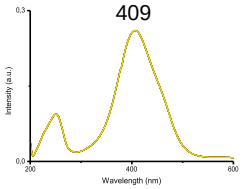
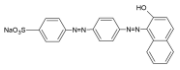
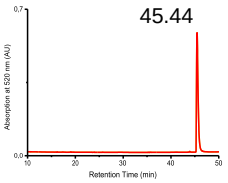
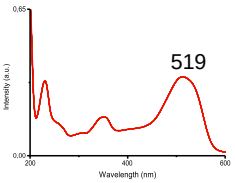
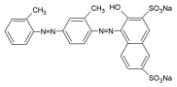
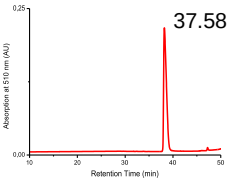
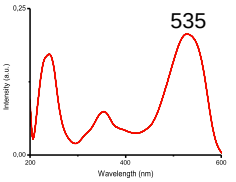
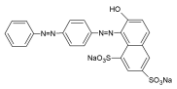
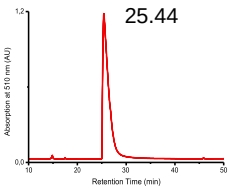
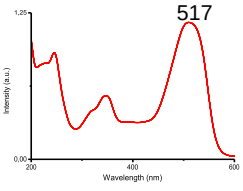
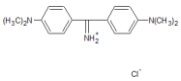
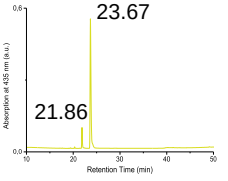
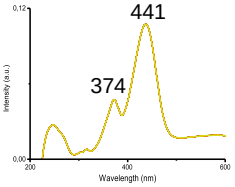
CI 13065 **Metanil Yellow** Acid Yellow 36			 31.03 min – 439 nm 33.05 min – 430 nm	375.38	31.03 and 33.05min: **MS** ***m/z* 352 [M-Na]$^-$** **MS^2** **352**→ 288 (50) (-64, SO_2) → 272 (15) (-80, SO_3) → 156 (75) (-196, $C_{12}H_{10}N_3$) **352**→ 325 (100) (-27, HCN) 272 (30) (-80, SO_3) → 260 (60) (-92, $^{\bullet}C_6H_6N$) → 156 (80) (-196, $C_{12}H_{10}N_3$)
CI 13080 **Orange IV** Acid Orange 5				375.38	**MS** ***m/z* 352 [M-Na]$^-$** **MS^2** **352**→ 288 (36) (-64, SO_2) → 272 (8) (-80, SO_3) → 156 (100) (-196, $C_{12}H_9N_3$)
CI 13090 **Azo Flavine 3R** Acid Orange 1 (acidified)		 1- 30.27 2- 35.68 3- 36.38 4- 37.95 5- 38.62 6- 39.95	  1- **441** 2- 401 3- 417 4- 448 5- **385 and 448** 6-390	449.37	**MS** (1,3,4) ***m/z* 397 [M-Na-NO+H]$^-$** (2) ***m/z* 442 (?)** (5,6) Not identified **MS^2** **397** → 333 (75) (-64, SO_2) → 317 (33) (-80, SO_3) → 156 (100) (-241, $C_{10}H_8N_3$+NO_2) **397** → 333 (100) (-64, SO_2) → 156 (75) (-241, $C_{10}H_8N_3$+NO_2) **397** → 333 (90) (-64, SO_2) → 156 (25) (-241, $C_{10}H_8N_3$+NO_2)
CI 13355 **Archil Red** Acid Red 74				394.34	**MS** ***m/z* 371 [M-Na]$^-$** **MS^2** **371**→ 325 (17) (-46, NO_2) → 307 (42) (-64, -SO_2) → 291 (32) (-80, SO_3) → 264 (100) (-107, C_7H_9N)

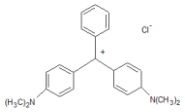
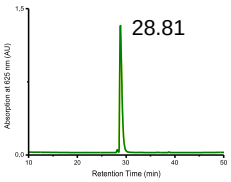
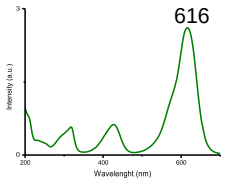
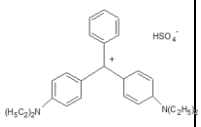
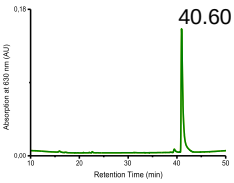
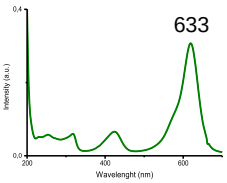
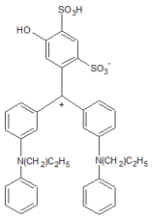
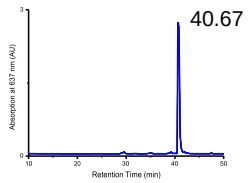
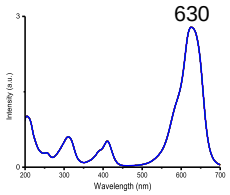
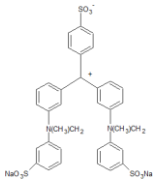
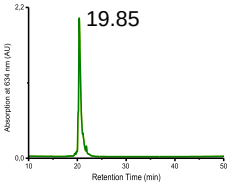
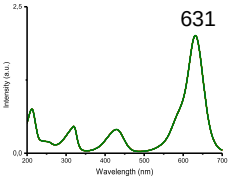
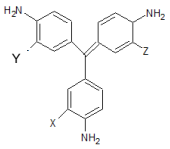
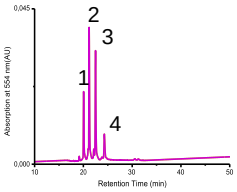
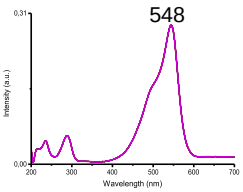
CI 14025 **Alizarin Yellow GGN** Mordant Yellow 1				309.21	**MS** ***m/z* 286 [M-Na]$^-$** **MS^2** **286→** 242 (100) (-44, CO_2)
CI 14270 **Tropaeolin O** Acid Orange 6				316.27	**MS** ***m/z* 293 [M-Na]$^-$** **MS^2** **293→** 275 (6) (-18, H_2O) → 213 (100) (-80, SO_3) → 171 (28) (-122, $C_6H_4O_2N$)
CI 14600 **Orange I** Acid Orange 20				350.32	**MS** ***m/z* 327 [M-Na]$^-$** **MS^2** **327→** 247 (100) (-80, SO_3) → 171 (94) (-156, $C_{10}H_7ON_2$)
CI 15510 **Orange II** Acid Orange 7				350.32	**MS** ***m/z* 327 [M-Na]$^-$** **MS^2** **327→** 247 (10) (-80, SO_3) → 171(100) (-156, $C_{10}H_7ON_2$)
CI 15620 **Fast Red AV** Acid Red 88			29.15 min:  40.10 min: 	400.38	**MS** ***m/z* 377 [M-Na]$^-$** **MS^2** **377→** 359 (20) (-18,H_2O) → 297 (6) (-80, SO_3) → 221 (100) (-156, $C_{10}H_6ON$) → 143 (22) (-234, $C_{10}H_6N_2$+ SO_3)
CI 15635 **Scarlet N for Silk** Acid Red 9			 40,17 min – 503 nm 40,86 min – 504 nm	400,38	**MS** (40.17 and 40.86min) ***m/z* 377 [M-Na]$^-$** **MS^2** **(40.17 min)** **377→** 221 (100) (-156, $C_{10}H_6ON$) **(40.86 min)** **377→** 221

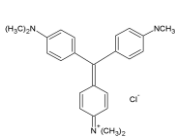
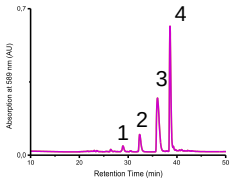
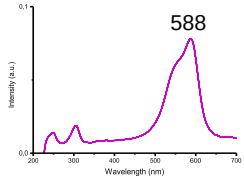
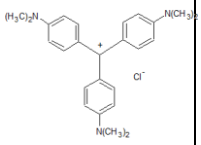
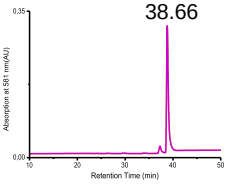
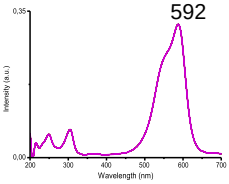
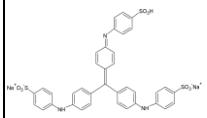
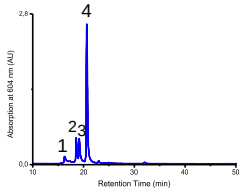
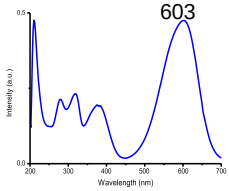
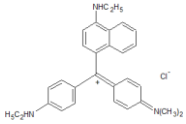
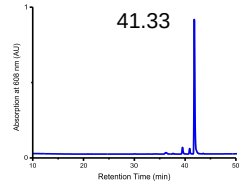
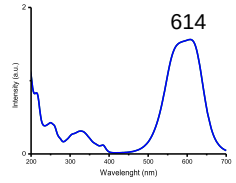
					(100) (-156, $C_{10}H_6ON$) → 206 (31) (-171, $C_{10}H_7ON_2$)
CI 15970 **Croceine Orange G** Acid **Orange** 2				350.32	**MS** ***m/z* 327 [M-Na]$^-$** **MS^2** **327→** 235 (100) (-92, C_6H_6N) → 207 (85) (-120, $C_6H_5N_2O$)
CI 16045 **Fast Red E** Acid **Red** 13				502.43	**MS** ***m/z* 457 [M-2Na+H]$^-$** **MS^2** **457→** 377 (77) (-80, SO_3) → 234 (9) (-223, $C_{10}H_7O+SO_3$)
CI 16050 **Ponceau 3RO** Acid **Red** 25				486.43	**MS** Peak (1) ***m/z* 537** Peak (2) ***m/z* 457 [M-2Na+H]$^-$** **MS^2** **457→** 429 (100) (-28, N_2) →349 (40) (108, N_2+SO_3)
CI 16100 **Ponceau 2G** Acid **Orange** 14		 1- 21.43 2- 28.33 3- 41.20	 1- 513 2- 492 3- 505	452.37	**MS** Peak (1) ***m/z* 435 (?)** Peak (2) ***m/z* 327** **[M-2Na-SO_3+H]$^-$** **MS^2** **327→** 171 (100) (-156, $C_6H_5+SO_3$)
CI 16150 **Ponceau 2R** Acid **Red** 26				480.42	**MS** ***m/z* 435 [M-2Na+H]$^-$** **MS^2** **435→** 417 (11)(-18, H_2O) → 355 (100) (-80, SO_3) → 337 (4) (-98, H_2O+SO_3) → 302 (14)(-133, $C_8H_9N_2$)
CI 16180 **Fast Red B** Acid **Red** 17				502.43	**MS** ***m/z* 457 [M-2Na+H]$^-$** **MS^2** **457→** 377 (100) (-80, SO_3) → 302 (22) (-155, $C_{10}H_7N_2$)

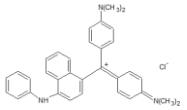
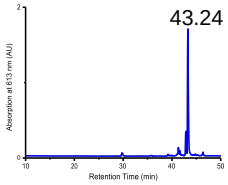
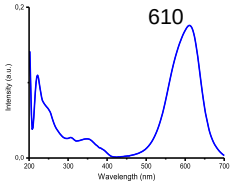
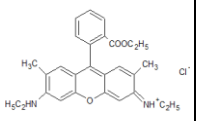
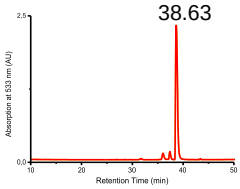
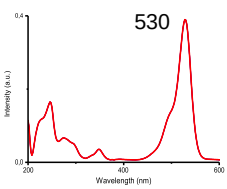
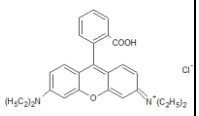
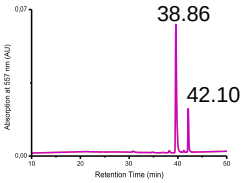
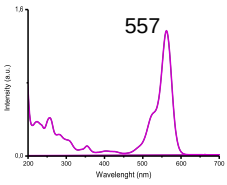
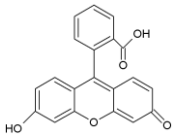
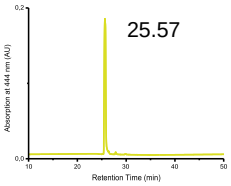
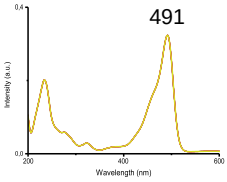
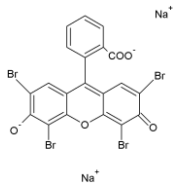
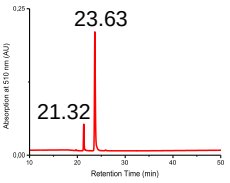
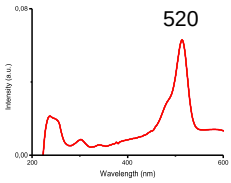
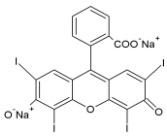
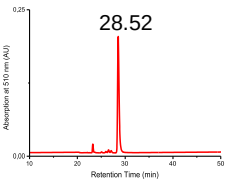
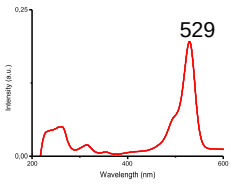
CI 16185 **Amaranth** Acid **Red** 27				604.47	MS ***m/z* 537 [M-3Na+2H]$^-$** MS^2 **537→** 519 (10) (-18, H_2O) → 473 (85) (64, -SO_2) → 457 (15) (-80, SO_3) → 317 (60) (-222,$C_{10}H_6NSO_3$)
CI 16230 **Orange GG** Acid **Orange** 10				452.37	MS ***m/z* 407 [M-2Na+H]$^-$** MS^2 **407→** 379 (-28, N_2)) → 327 (-SO_3) → 302 (-$C_6H_5N_2$)
CI 16250 **Crystal Ponceau 6R** Acid **Red** 44				502.43	MS ***m/z* 537** Not identified
CI 16255 **Cochineal Red A** Acid **Red** 18				604.47	MS ***m/z* 537 [M-3Na+2H]$^-$** MS^2 **537→** 509 (100)(-28, N_2) → 457 (10) (-80, SO_3) → 429 (30) (-108, CO+ SO_3)
CI 16290 **Ponceau 6R** Acid **Red** 41		 1- 7.02 2- 14.90 3- 16.42	 1- 517 2- 515 3- 510	706.52	MS ***m/z* 617 [M-4Na+3H]$^+$** MS^2 617→ 589 (100), (-28, N_2) →509 (50) (-108, CO+SO_3) →445 (25)(-172, $C_{10}H_8N_2O$)
CI 17200 **Fast Acid Magenta B** Acid **Red** 33				467.38	MS ***m/z* 422 [M-2Na+H]$^-$** MS^2 **422→** 358 (36) (-64, SO_2) → 317 (59) (-105, $C_6H_5N_2$) → 249 (100) (-173, $C_9H_7N_3O$)
CI 18050 **Amido Naphthol Red G** Acid **Red** 1			17.40 min: 	509.42	MS **17.40 min.:** ***m/z* 422 [M-2Na-$COCH_2$+H]$^-$** **18.60 min.:** **m/z 464 [M-2Na+H]$^-$** MS^2 **422→** 358 (42) (-64, SO_2) 342 (20) (-80, SO_3)

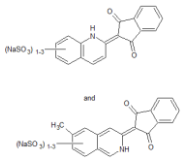
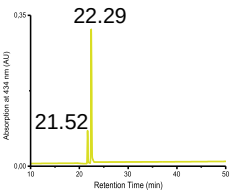
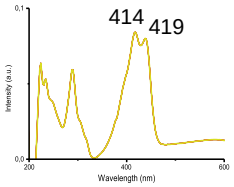
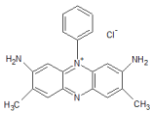
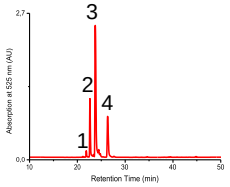
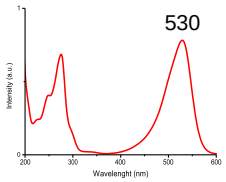
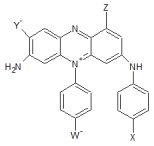
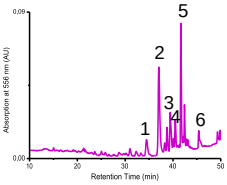
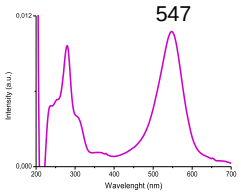
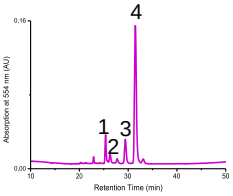
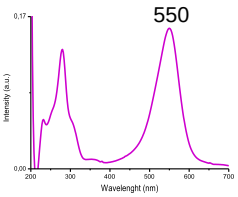
			18.60 min: 		→ 317 (49) (-105, $C_6H_5N_2$) → 249 (100) (-173, $C_9H_7N_3O$) **464→** 359 (100)(-105, $C_6H_5N_2$)
CI 18055 **Azo Fuchsine 6B** Acid **Violet** 7		 1- 13.23 2- 16.12 3- 17.88	 1-531 2-541 3-530	566.47	**MS** (1) **m/z 479 [M-2Na-CH$_3$CO+H]$^-$** (2) ***m/z* 521 [M-2Na+H]$^-$** **MS^2** **479→** 331 (100)(-148, $C_8H_8N_2$) → 249 (95) (-230, $C_8H_8N_3$+SO_2) 521→ 493 (9) (-28, CO) → 373 (34) (-148, $C_8H_8N_2$) → 359 (100) (-162, $C_8H_8N_3$)
CI 18820 **Flavazine L** Acid **Yellow** 11			 18.53 – 406 29.59 – 408	380.35	**MS** ***m/z* 425/427** Not identified (should be a chlorinated compound: the relative abundance of the ion presents the characteristic ration of a chloride atom ^{35}Cl:^{37}Cl, 1:3)
CI 19140 **Tartrazine** Acid **Yellow** 23				534.36	**MS** ***m/z* 533 [M-H]$^-$** ***m/z* 489 [M-2Na+H]$^-$** **MS^2** **533→** 511 (100) (-22, -Na+H) → 489 (56) (-44, CO_2)
Disazo					
CI 20470 **Amido Black 10B** Acid **Black** 1				616.49	**MS** ***m/z* 571 [M-2Na+H]$^-$** **MS^2** **571→** 507 (100) (-64, SO_2) → 479 (5) (-C_6H_6N)

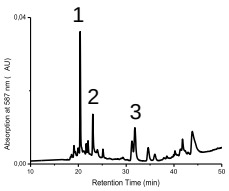
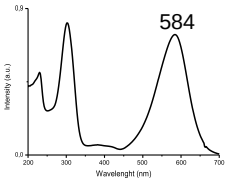
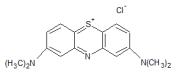
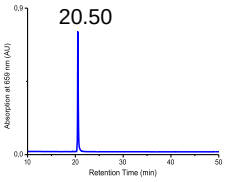
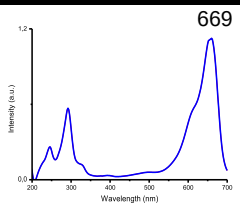
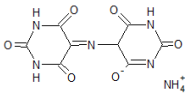
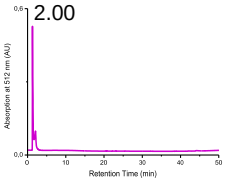
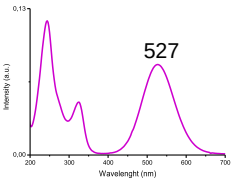
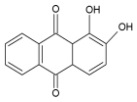
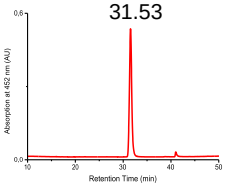
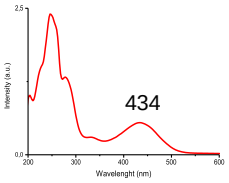
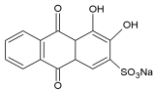
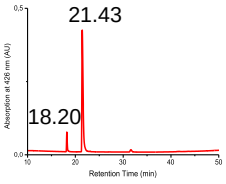
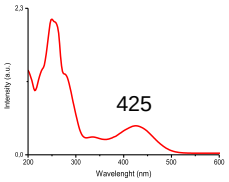
CI 21000 **Vesuvine BA** Basic **Brown** 1		 1- 14.54 2- 16.96 3- 27.27	 1- 460 2- 459 3- 463	419.31	N.I.
CI 22120 **Congo Red** Direct **Red** 28				696.66	**MS** ***m/z* 651 [M-2Na+H]$^-$** **MS^2** **651→** 587 (8)(-64, SO_2) → 571 (100) (-80, SO_3)
CI 24890 **Brilliant Yellow** Direct **Yellow** 4				624.55	**MS** ***m/z* 579 [M-2Na+H]$^-$** **MS^2** **579→** 515 (8)(-64, SO_2) → 499 (100)(-80, SO_3) → 458 (34)(-121,$C_6H_5N_2O$)
CI 26900 **Cloth Scarlet** Acid **Red** 151				454.43	**MS** ***m/z* 431 [M-Na]$^-$** **MS^2** **431→** 367 (56) (-64, SO_2) → 275 (100) (-156,$C_{12}H_{12}$)
CI 27200 **Wool Red B** Acid **Red** 115				584.53	**MS** ***m/z* 539 [M-2Na+H]$^-$** **MS^2** **539→** 521 (22)(-18, H_2O) → 459 (100) (-80, SO_3)
CI 27290 **Cotton Scarlet** Acid **Red** 73				556.48	**MS** ***m/z* 511 [M-2Na+H]$^-$** **MS^2** **511** → 483 (75), (-28, N2) → 431 (20) (-80, SO_3) → 403 (10) (-108, N_2+SO_3) → 302 (100) (-209, $C_{12}H_9N_4$)
Diphenylmethane					
CI 41000 **Auramine O** Basic **Yellow** 2			 1- 433 2- 374 and 441	303.83	**MS** (1) ***m/z* 254 (?)** (2) ***m/z* 268 [M]$^+$** **(3) m/z 255 (?)** (4) ***m/z* 269 (?)** **MS^2** **268→** 235 (-CH_3) → 147 (-$C_8H_{11}N$)

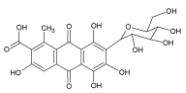
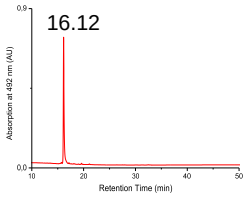
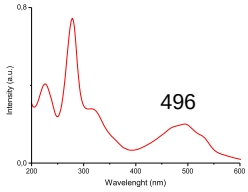
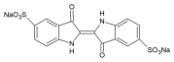
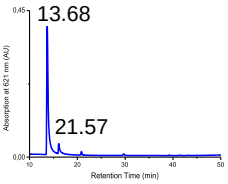
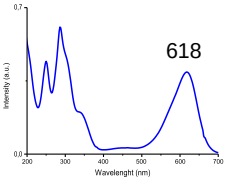
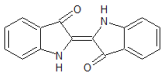
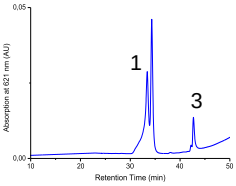
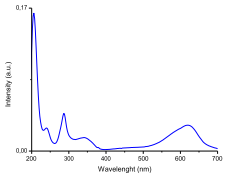
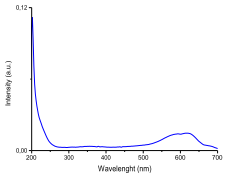
					→ 122 ($-C_9H_{11}N_2$) **269→** 148 (100) (-121,$C_8H_{11}N$)
Triarylmethane					
CI 42000 **Malachite Green** Basic **Green** 4				329.46	**MS** ***m/z* 329 [M]$^+$** **MS^2** **329→** 313 (78)(-16,CH_4) → 314 (60) (-15, $^\bullet CH_3$) → 286 (28) (-43, NC_2H_5) → 208 (100)(-121,$C_8H_{11}N$) → 251 (40), (-78, C_6H_6)
CI 42040 **Brilliant Green** Basic **Green** 1				482.63	**MS** ***m/z* 385 [M]$^+$** **MS^2** **385→** 355 (20) (-30, C_2H_6) → 356 (10) (-29, $^\bullet C_2H_5$) → 341 (100) (-44, C_3H_8)
CI 42051 **Patent Blue V** Acid **Blue** 3				685.83	**MS** ***m/z* 667(-)** or ***m/z* 669(+)**
CI 42075 **Light Green SF Bluish** Acid **Green** 6				764.48	**MS** ***m/z* 373** N.I.
CI 42510 **Fuchsine** Basic **Violet** 14	 Pararosanilin: **X**=H, **Y**=H, **Z**=H Rosaniline: **X**=CH_3, **Y**=H, **Z**=H ----------: **X**=CH_3, **Y**=CH_3, **Z**=H New Fuchsine:	 1- 19.74 2- 20.82 3- 21.98 4- 25.57	 1- 547 2- 548 3- 551 4- 556	288.37 302.39 316.42 330.44	**MS** (1) ***m/z* 288 [M]$^+$** (2) ***m/z* 302 [M]$^+$** (3) ***m/z* 316 [M]$^+$** (4) ***m/z* 330 [M]$^+$** **MS^2** **288→** 273 (8) (-15, $^\bullet CH_3$) → 195 (100) (-93, C_6H_7N) → 168 (6) (-120, $C_7H_8N_2$) **302→** 287 (10) (-15, $^\bullet CH_3$) → 209 (100) (- 93, C_6H_7N) → 195 (46) (-107,C_7H_9N) **316→** 301 (13) (-15, $^\bullet CH_3$) → 223 (42) (-93,C_6H_7N)

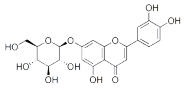
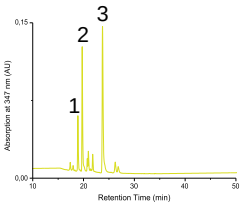
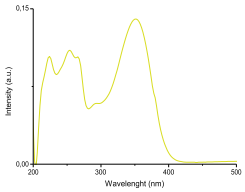
	X=CH_3,**Y**=CH_3, **Z**=CH_3				→ 209 (100) (-107,C_7H_9N) **330**→ 315 (8) (-15, •CH_3) → 223 (100) (-107,C_7H_9N) → 208 (12) (-122, C_7H_9N+•CH_3)
CI 42535 **Methyl Violet** Basic **Violet**		 1- 28.89 2- 32.35 3- 36.00 4- 38.57	 1- 578 2- 574 3- 580 4- 588	393.97	**MS** (1) ***m/z* 330 [M]$^+$** (2) ***m/z* 344 [M]$^+$** (3) ***m/z* 358 [M]$^+$** (4) ***m/z* 372 [M]$^+$** **MS^2** **344**→ 328 (80) (-16,CH_4) → 329 (100) (-15, •CH_3) →237 (60) (-107,C_7H_9N) →223 (14) (-121,$C_7H_9N_2$) **358**→ 342 (100) (-16,CH_4) → 343 (80) (-15, •CH_3) → 251 (9) (-107, C_7H_9N) → 237 (10) (-121,$C_7H_9N_2$) **372**→ 357 (40) (-15, CH_3) → 251 (16) (-121,$C_7H_9N_2$)
CI 42555 **Crystal Violet** Basic **Violet**				407.98	**MS** ***m/z* 372 [M]$^+$** **MS^2** **372**→ 357 (60) (-15,CH_3) → 328 (6) (-44,C_2H_5N) → 251 (17) (-121,$C_8H_{11}N$)
CI 42780 **Methyl Blue** Acid **Blue** 93		 1- 16.27 2- 18.55 3- 19.13 4- 20.63	 1- 612 2- 606 3- 601 4- 603	799.80	**MS** ***m/z* 754 [M-2Na+H]$^-$** ***m/z* 376,5 [M-2Na]$^{2+}$**
CI 44040 **Victoria Blue R** Basic **Blue** 11				458.97	**MS** ***m/z* 422 [M]$^+$** **MS^2** **422**→ 394 (30) (-28, C_2H_4) → 301(100) (-121,$C_8H_{11}N$) → 272 (35) (-150,$C_{10}H_{15}N$)

CI 44045 **Victoria Blue B** Basic **Blue** 26				506.08	**MS** ***m/z* 470 [M]$^+$** **MS^2** **470→** 455 (11) (-15,$^\bullet$CH$_3$) → 454 (10) (-16, CH$_4$) → 349 (100) (-121,C$_8$H$_{11}$N)
Xanthene					
CI 45160 **Rhodamine 6G** Basic **Red** 1				479.02	**MS** ***m/z* 443 [M]$^+$** **MS^2** **443→** 415 (100) (-28,C$_2$H$_4$) **MS^3** **415→** 386 (100)(-29, C$_2$H$_5$) →341 (26) (-74, HCOC$_2$H$_5$)
CI 45170 **Rhodamine B** Basic **Violet** 10			 38.86 min – 557 nm 42.10min - 588 and 622 nm	479.02	**MS** ***m/z* 443 [M]$^+$** **MS^2** **443→** 415 (15) (-30,C$_2$H$_6$) → 399 (100) (-44,CO$_2$)
CI 45350 **Uranine A** Acid **Yellow** 73				332.30	**MS** ***m/z* 333 [M+H]$^+$** **MS^2** **333→** 315 (8) (-18,H$_2$O) → 305 (33) (-28,CO) → 287 (100)(-46,H$_2$O+CO) → 272 (22) (-62,H$_2$O+CO$_2$)
CI 45380 **Eosine A** Acid **Red** 87			 21.32 min – 514 nm 23.63 min – 520 nm	691.88 (2)	**MS** (1) **m/z 569 (N.I.)** (2) ***m/z* 647 [M-2Na+H]$^-$** **MS^2** **647→** 603 (10) (-44,CO$_2$) → 523 (100) (-124, Br+CO$_2$H) → 443 (5) (-204, Br$_2$+CO$_2$H)
CI 45430 **Erythrosine** Acid **Red** 51				879.86	**MS** ***m/z* 835 [M-2Na+H]$^-$** **MS^2** **835→** 791 (12) (-44,CO$_2$) → 663 (100)(-172,I+CO$_2$H) → 537(40)(-298,I$_2$+CO$_2$H))

Quinoline					
CI 47005 **Quinoline** Acid Yellow 3			 21.52 min- 421 and 439 nm 22.29 min- 414 and 419 nm	477.38	**MS** 21.52 and 22.29 min.: ***m/z* 352** N.I.
Azine					
CI 50240 **Safranine T** Basic Red 2		 1- 21.85 2- 22.62 3- 23.73 4- 26.32	 1- 533 2- 536 3- 530 4- 528	350.84	**MS** **m/z 443** N.I.
CI 50245 **Mauveine** Basic Dye	 Mauveine A: **X**=CH_3, **Y**=CH_3, **Z**=H, **W**=H Mauveine B: **X**=CH_3, **Y**=CH_3, **Z**= CH_3, **X**=H Mauveine C: **X**=CH_3, **Y**=CH_3, **Z**=CH_3, **W**=CH_3 Mauveine C_{25}A: **X**= H, **Y**=CH_3, **Z**=H, **W**=H	 1- 37.08 2- 38.75 3- 39.51 4- 40.58 5- 41.72 6- 42.32	 1- 552 2- 543 3- 546 4- 541 5- 547 6- 539	391.2 405.3 419.3 377.2	**MS** ***m/z* 391 $[M]^+$** ***m/z* 405 $[M]^+$** ***m/z* 419 $[M]^+$** ***m/z* 377 $[M]^+$** **MS^2** **391→** 375 (100) (-16,NH_2) → 360 (28) (-31,NH_2+CH_3) → 284 (42) (-107, C_7H_9N) **405→** 344 (4) (-61,$C_3H_{11}N$) → 298 (91) (-107,C_7H_9N) **419→** 403 (82) (-16,NH_2) → 389 (50) (-30,C_2H_6) →327 (7) (-92, C_6H_6N) → 299 (100)(-120, $C_8H_{10}N$) **377→** 361 (100) (-16, NH_2) →284 (35) (-93,C_6H_7N) → 270 (56) (-107,C_7H_9N)
CI 50245 Mauveine (fiber)		 1- 25.35 2- 26.28 3- 29.42 4- 31.47	 1- 557 2- 567 3- 558 4- 555		

CI 50420 **Nigrosine** Acid **Black** 2	unknown	 1- 20.35 2- 21.71 3- 31.80	 1- 584 2- 565 3- 439	(?)	N.I.
Thiazine					
CI 52015 **Methylene Blue** Basic **Blue** 9				319.90	**MS** ***m/z* 284 $[M]^+$** **MS^2** **284**→ 269 (51) (-15,$^{\bullet}CH_3$) → 241 (4) (-43,NC_2H_5)
Aminoketone/Lactone					
CI 56085 **Murexide** Mordant Dye				284.19	**MS** ***m/z* 266 $[M]^-$** **MS^2** **266**→ 223 (100) (-43,NCHO) → 180 (20) (-86, $(NCHO)_2$)
Anthraquinone					
CI 58000 **Alizarin** Mordant **Red** 11				240.20	**MS** ***m/z* 239 $[M-H]^-$** **MS^2** **239**→ 211 (100) (-28,CO)
CI 58005 **Alizarin Red S** Mordant **Red** 3			 18.20 min - 421 nm 21.43 min - 425 nm	342.30	**MS** ***m/z* 319 $[M-Na]^-$** **MS^2** **319**→ 255 (13) (-64,SO_2) → 239 (100) (-80,SO_3)

Cochineal (acidified)	 Carminic acid (principal chromophore)			491	**MS** Carminic acid: ***m/z* 491 [M-H]$^-$** **MS^2** **491**→ 447 (100) (-44,CO_2) → 429 (60) (-62,CO_2+H_2O) →357 (20) (-90, $C_3O_3H_4$) → 327 (7) (-164, -glu) (for more information see in [5])
Indigoid					
CI 73015 **Indigoid** Acid **Blue** 74			 13.68 min – 618 nm 21.57 min – 613 nm	466.35	**MS** For the both peaks: ***m/z* 443 [M-Na]$^-$** ***m/z* 421 [M-2Na+H]$^-$** **MS^2** **443**→ 415 (5) (-28,CO) → 379 (19) (-64,SO_2) → 363 (100) (-80,SO_3) **421**→ 341 (100) (-80,SO_3)
Indigo (natural dye)		 1- 33.38 2- 34.30 3- 42.67	1 and 2- 619 and 618  3- 617 	262	**MS** ***m/z* 263 [M+H]$^-$** **MS^2** **263**→ 235 (30) (-28, CO) → 219 (100) (-44, $CONH_2$)

Flavonoids					
Weld (Reseta luteola)	 Luteolin-7-*O*-glucoside (principal chromophore)	 1- 19.73 2- 20.93 3- 23.73	 1- 350 2- 349 3- 351	286 449	**MS** Luteolin-7-O-glucoside: ***m/z* 448 $[M+H]^+$ **MS^2** Luteolin-7-*O*-glucoside: **449→** 287 (100) $Y_0^+ \rightarrow$ 269 (20) $[M+H-H_2O]^+$, 259 (12) $[M+H-CO]^+$, 245 (20) $[M+H-C_2H_2O]^+$, 241(40) $[M+H-H_2O-CO]^+$, 219 (12) $[M+H-C_2H_2O-C_2H_2]^+$, 203 (8) $[M+H-C_2H_2O-C_2H_2O]^+$, 179 (8) $^{0,4}\mathbf{B}^+$, 161 (18) $^{0,4}\mathbf{B}^+$-H_20, 153 (100) $^{1,3}\mathbf{A}^+$, 137 (10) $^{0,2}\mathbf{B}^+$, 135 (8) $^{1,3}\mathbf{B}^+$ (MS of one principal chromophore. For more information see in [5])

Apendix IV – Persian Carpet Catalogue

Persian Carpet T107

Weaver: Mr. Hagop Kapoudjin

Portugal, 20th century

Silk warp and weft, executed with very fine Persian asymmetrical knots

1,80 x 1,26 m

Lisbon, Museu Calouste Gulbenkian

Ottoman Turkey style composed by palmettes enriched with golden metal-wrapped silk threads. The deep dark red homogeneous background (L= 18,30 ± 0,10; a*=17, 86 ± 0,03; b*= 6,55 ± 0,11) was identified as scarlet N for Silk (CI 15635) and cotton scarlet (CI 27290). The yellow and beige (L= 50.66 ± 0,08; a*= 3.87 ± 0; b*= 26.19 ± 0.02) was identified as azo flavine 3R (CI 13090) and orange IV (CI 13080). The blue (L= 20.94 ± 0.12; a*= -2.12 ± 0.02; b*= 5.54 ± 0.13) was not identified and the green: (L= 35.68 ± 0.07; a*= 1.21 ± 0.04; b*= 22.85 ± 0.07) was obtained by a mixture of yellow and blue dyes.

Appendix references

[1] Paine, C., *Perkin Centenary London, 100 years of synthetic dyestuffs,* Pergamon Press, Great Britain (1958) 15-17.

[2] Garfield, S., *Mauve, How One Man Invented a Colour that Changed the World,* Faber and Faber, London (2000).

[3] Schweppe, H., *Practical Information for the Identification of Early Synthetic Dyes, Practical hints on Dyeing with Early Synthetic Dyes,* Conservation Analytical Laboratory, Smithsonian Institute, Washington DC USA.

[4] Serrano, A., *Cochineal, a Precious Source of Red, Cochineal Dyes Identification by High Performance Liquid Chromatography with Diode Array Detector and Principal Component Analysis*, Master thesis dissertation, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisbon (2010).

[5] Marques, R., Sousa, M.M., Oliveira, M.C. and Melo, M.J., 'Characterization of weld (*Reseda luteola L.*) and spurge flax (*Daphne gnidium L.*) by high-performance liquid chromatography-diode array detection-mass spectrometry in Arraiolos historical textiles', *Journal of Chromatography A* **1216** (2009) 1395-1402.