Stability of natural dyes under light emitting diode lamps

Research highlights

- The suitability of LEDs illumination for historical textiles is considered.
- The effects of thee LED lamps on silk samples dyed with six natural dyes are discussed.
- Small colour changes related to exposure to LEDs light are associated to relevant variations of the original level of the colouring molecules.

Stability of natural dyes under Solid State Lighting

L. Degani[1], M. Gulmini[1*], G. Piccablotto[2], P. Iacomussi[3], D. Gastaldi[4], F. Dal Bello[4], O. Chiantore[1]

[1] Università degli Studi di Torino, Dipartimento di Chimica, via Giuria, 5 - 10125 Torino, Italy

[2] Politecnico di Torino, Dipartimento Architettura e Design, Laboratorio LAMSA, corso M. D'Azeglio, 42 - 10125 Torino, Italy

[3] Istituto Nazionale di Ricerca Metrologica, Strada delle Cacce, 91 - 10135 Torino, Italy

[4] Università degli Studi di Torino, Dipartimento di biotecnologie molecolari e scienze per la salute, Via Nizza,52 - 10124 Torino, Italy

* Corresponding author: monica.gulmini@unito.it

Abstract

The exhibition in museum of archaeological and historical coloured textiles must keep into consideration the possible photo-degradation of dyes. In the last decade, Light Emitting Diode lamps (LED) have been extensively replacing other light sources; nevertheless few studies on the influence of LEDs on degradation of natural dyes are available.

In this work, the colour fading of silk samples dyed with several natural dyes (containing flavonoids and anthraquinones) and exposed to three different white LEDs is considered. The fading at the end of the exposure experiment was evaluated by measuring the variations induced by the LEDs on the colour coordinates of the samples and by investigating the variation of the concentration of the dyes by high performance liquid chromatography coupled with photo-diode array and mass spectrometric detectors.

The information obtained gives an in depth picture of the fading in terms of the actual damage potential of LEDs on natural dyes and it is relevant for selecting lamps for display cases.

1. Introduction and research aims

Natural dyes are among the most fugitive materials and were used for decorative purposes in the past. The conservation and the display of historical and archaeological objects requires particular attention if natural dyes are present, and specific problems must be addressed. Lighting in museums must render the original colours of the displayed objects, and also must meet the conservation issues for photosensitive molecules. Several studies on the light sensitivity of natural dyes employed for colouring fabrics and yarns are available, and they highlighted that the stability of the natural dye is affected by a number of factors [1-6].

Nevertheless the chemical structure of the molecules that are responsible for the colour is an important intrinsic factor that influences light-fastness. Both skeleton structures of the different chemical families of dyes and the position of auxochrome substituents determine the degradation pathway upon light exposure. External factors, such as temperature and humidity, can also affect the degradation reactions [2-5], although the energy distribution and the intensity of the illumination are the principal external factors that must be considered when displaying historical textiles [6-9].

In the last decade, the use of white light emitting diodes (LEDs) has largely increased, firstly because they are among the most energy-saving light sources, and also because of the negligible UV and infrared components in their emission spectra. White LEDs are therefore replacing fluorescence and incandescence lamps in many museums and art galleries, but only a few systematic studies on the effects of white LED emission on the colour of historical textiles are available [10, 11].

The work aims at evaluating the suitability of LED lighting for illuminating historical textiles in display cases. Both the colour changes (mainly in terms of variation of colour coordinates of the samples) and the modifications that occurred in the concentration of the various colouring molecules were determined on silk cloths prepared in the laboratory to this aim.

Samples were obtained by dyeing silk clothes with plants (or insects) selected among the most widely used materials for dyeing in the past [12, 13]. Dyes from two kinds of chemical families were

considered: flavonoids (from weld, old fustic, logwood and brazilwood) and anthraquinones (cochineal and madder). The samples represent therefore common situations that can be encountered when displaying ancient coloured textiles. Such textiles also allowed us to investigate the response of the different types of molecular structures under the LED lighting.

The following samples were considered here for the investigation:

a) yellow silk dyed with weld or old fustic, b) blue-violet silk dyed with logwood and c) red silk dyed with brazilwood, cochineal or madder. The samples were exposed to three types of white LED lamps, with the same white light emission technology (i.e. blue LED with phosphor coating), but with different Correlated Colour Temperature (CCT). The provided light dose, at the end of the experiment, was equivalent to more than 1000 years under museum controlled lighting of 50 klx h/year light dose as requested by Italian Cultural Heritage Preservation Act [14]: such an high dose was chosen for our experiments in order to enhance the possibility of detecting differences among the three lamps in terms of fading of the exposed textiles, and for obtaining significant colour variations that would help a direct comparison between colourimetric and chromatographic data. Samples dyed with weld, old fustic, cochineal and madder were in fact considered in order to determine the variation induced by the LED in the concentration of the colouring molecules. High performance liquid chromatography coupled with a photo-diode array and a mass spectrometric detector (HPLC-PDA-MS) was used to this aim, by analysing the dyes extracted from the fabrics at the beginning and at the end of the fading experiment.

2. Methods

2.1. Materials and instruments

Hydrochloric acid (HCl), methanol (MeOH), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), aluminum potassium sulfate dodecahydrate (alum), formic acid (FA); dimethylformamide (DMFA), acetonitrile (CH_3CN) alizarin, carminic acid, apigenin, luteolin, morin, kaempferol and purpurin were purchased from Sigma-Aldrich (Milano, Italy).

Madder (*Rubia tinctorum* L. roots), cochineal (*Dactylopius coccus* Costa dried insects), weld (dried leaves and stems of *Reseda luteola* L), old fustic (extract of *Chlorophora tinctoria* L.) and logwood (extract of *Haematoxylum campechianum* L.) were purchased from Kremer Pigmente (Aichstetten, Germany). Brazilwood (extract of *Caesalpinia echinata* Lamarck) was purchased from Critt Horticole (Rochefort/Mer, France). The structures of the main colouring chemical species associated with the considered natural dyeing materials are shown in Figure 1

The illuminance levels in the fading experiment were checked by a Gigahertz-Optik P9710 class A luxmeter [15] and the reflectance spectra of the samples during the exposition to LEDs light were collected by a UV-Vis-Nir Perkin Elmer Lambda 900 double beam spectrometer measuring the spectral reflectance of each sample with a dedicated measurement procedure with: measurement range - 250-2500 nm, 1 nm step, 8° of incidence and diffuse reflectance (8/d). As the samples showed a diffuse behaviour, the specular component was also included in the measurements. The CIE 2° standard observer and the equi-energy spectrum (illuminant E) were used for calculating the colourimetric data. An Ultimate 3000 Dionex HPLC instrument coupled both with a PDA detector and a LTQ-Orbitrap analyser (Thermo Scientific, Milan, Italy) was used for the target molecules. The separation (20 μl injected) was carried at 30°C. The column (C18-bonded silica, 150×2.1 mm, 3 μm particle size from Phenomenex, Torrence, California, USA) was eluted at a flow rate of 0.2 mL/min with CH_3CN (A), and 0,05% (v/v) FA (B) with gradient elution from 95% A, 5% B to 5% A, 95% B in 30 min. The mass spectrometer was run in positive and negative ion mode and data were processed with the Excalibur 2.0.7 Software.

For the Electron Spray Ionization (ESI) source, the temperature was set at 275 °C, the ion spray voltage at +4.5 kV. For the Atmospheric Pressure Chemical Ionization (APCI) source, the voltage was 4.5kV; capillary temperature 275°C, discharge current 5μA and capillary voltage 10V.

Mass spectra were collected in the range 50–900 *m/z* in full scan mode (positive or negative) and in a range comprised between the precursor ion m/z value and the relative ion trap cut-off

acquiring MS^2 spectra. MS^2 collision energy was generally chosen in order to maintain about 10% of the precursor ion.

2.2. Preparation of the samples

The samples were obtained from a taffeta fabric (about 25 g), which was purchased already spun and scoured. It was divided into 6 cloths, which were mordanted with alum and dyed with madder, cochineal, weld, old fustic, brazilwood or logwood. The dyeing procedures were performed following the indication given in the literature about ancient dyeing [12,13]. The cloths were previously soaked in deionised water and then mordanted for 30 min in a boiling solution of alum (5 g/L, 500 ml). After this treatment they were left to dry protected from light. In the meanwhile, the dye-baths were prepared by treating 5 g of the dyeing materials for 2 h at 90°C in 50 mL of deionised water. When treating cochineal, the dried insects were soaked in water for 24 h before heating the system. The solutions were filtered and cooled at room temperature. One cloth was immersed into each of the prepared dye-baths, and heated at 90 °C for 30 min. After cooling, the dyed silk clothes were recovered from the baths, rinsed with water and dry at room temperature and protected from direct light.

2.3. Fading experiment

The six silk cloths were cut into smaller sections of 2.5x2.0 cm and arranged in a 10X10 cm^2 cardboard. Four sets of samples, each displaying all the six hues, were obtained. One set was kept in the dark as unexposed reference, the other three were positioned respectively inside three dedicated fading boxes, designed and built specifically for this experiment. Each box also contained a data-logger for light dose and microclimate measurements. The LED lamp was mounted on the top of each box, with the heat sink outside the box in order to avoid perturbation of the temperature. The three boxes operated in a laboratory with microclimatic parameters set at T_{air}=23±2 °C and RH=50±5 %. These parameters were the same as those recorded inside the boxes. Each box was equipped with one of the three investigated light sources: NW-LED, WW-LED1 and WW-LED2. For each light source, the code naming is related to the general photo-

radiometric characteristics reported in Table 1. In particular, NW stands for Neutral White, an attribute of sources with CCT around 4000K, whereas WW stands for Warm White, which is the attribute of sources with CCT lower than 3000K. The normalised spectral intensity distribution of the light sources is shown in Figure 2. The stability of the lamps and the light dose were checked regularly during the overall duration of the fading experiment, the light dose at the end of the experiment was accurately calculated taking in account the luminous flux depreciation of each lamp. In particular, the final light dose was 64.9 Mlx h for NW-LED, 66.1 Mlx h for WW-LED1 and 67.5 Mlx h for WW-LED2. Figure 3 shows the samples before and after the fading experiment (NW-LED).

A CIE publication about light induced damage [16] identifies madder, old fustic and cochineal as high responsivity materials (Blue Wools 2 and 3) and available standards [14, 17] suggest an annual dose of 50 klx h /year for such sensitive materials. Therefore the luminous dose provided here is equivalent to an exposition of about 1300 years at 50 klx h /year. This temporal forecast is clearly the worst case and is based only on light dose data suggested in these standards. Nevertheless it is functional to the main goal of this study, which is to highlight the damage potential of LED lighting on natural dyes.

2.4. Colourimetric measurements

The spectral reflectance of each sample was measured at regular intervals during the whole duration of the fading experiment (9 months of light exposure). Instruments and setups were those described in 2.1. The colour coordinates CIE L*a*b* and CIE L*C*h* were calculated after each measurement.

The samples were centred with respect to the portal of the integrating sphere of the double beam spectrometer according to an alignment frame specifically prepared for each sample in order to reduce repositioning errors. Before each measurement session, the following procedure for spectral reflectance was followed: 20 minutes instrument warm-up, internal calibration and grating alignment, baseline acquisition, a reference Spectralon sample was measured for performance

verification at the beginning and at the end of the measurement trial. Repeatability and reproducibility performance of the instrument were taken into account in the evaluation of the uncertainty for spectral reflectance measurements.

During the characterisation of one set, the other two were still exposed to the light, and the exposure time of each set was calculated and recorded. The light exposure during the measurement was not taken into account in the final calculations, as it was considered negligible over the whole light dose.

For each set of samples, reflectance and colourimetric data at the end and at the beginning of the fading experiment were compared. Changes in colour were evaluated using CIELab ΔE and CIEDE2000 values. The measurement uncertainty on CIE L*a*b* and ΔE was evaluated by propagating the uncertainty on the spectral reflectance values calculated following the Guide for uncertainty evaluation of the International Organisation for Standardisation (ISO) [18] and also taking into account the metrological characterisation of the spectrometer. The propagation of the uncertainty in evaluating ΔE is complex because of the inner correlations between XYZ, i.e. the colourimetric components calculated from the spectral reflectance, and L*a*b* values. The application of a high level theoretical model for uncertainty evaluation is out of the scope of this work, so we choose the easy option of considered the variables fully correlated, setting as measurement uncertainty for all samples, the highest value calculated by combining uncertainty value on ΔE. This value was estimated as 1 unit of ΔE. This approach is really cautionary, as it overestimates the uncertainty. Nevertheless it can be considered appropriate for the purpose of this work, especially because the Just Noticeable Difference (JND), according to the CIE 2004 report [16], corresponds to colour variation of ΔE = 1.6.

2.5. Extraction procedure

The silk samples after the exposure to LED light and those of the non-exposed reference set were treated in order to extract the dyes. The most suitable extraction procedure was selected after some preliminary tests slightly modifying the extraction methods reported in the literature [19,20].

In particular, two different “mild” methods of extraction, i.e.: 0.1% EDTA in water/DMF (1:1 v/v) and FA/MeOH (1:19 v:v), were tested [19]. Unfortunately, none of these reagents achieved an exhaustive extraction of the dyes from the fabric, and samples were still coloured even after having repeated the extraction treatment up to three times. On the contrary, the extraction based on H_2O:MeOH:HCl [21] caused the hydrolysis of the textile support and ensured the quantitative extraction of all the colouring species. It is known that this method causes the cleavage of glycosidic bonds, nevertheless it enables the possibility of comparing the levels of the aglycones which have not been degraded during the fading experiment under the different LED lamps.

Brazilein and haematein, which are formed from colourless brazilin and haematoxylin, become increasingly unstable below pH 5 and produce a number of by-products as a consequence of excessive oxidation [22-28]. This prevents the accurate estimation of the percentage of the coloured species that remained after the fading experiment, therefore the HPLC-PDA-MS insight was not performed on the samples dyed with brazilwood and logwood.

The extraction was performed as follows: 2 mg of the silk sample were extracted in a reaction tube with 2.5 ml of H_2O:MeOH:HCl (1:1:2 v:v:v) for 30 minutes in a boiling water bath. All the samples were treated in parallel, in order to ensure common conditions for the extraction. The hydrolysed samples were cleared in a centrifuge (10 min at a relative centrifugal force of 36 000). The clear supernatants were dried by gently blowing nitrogen on the solutions. Then the residues were dissolved in 600 µl of H_2O/MeOH (1/1, v/v) and injected in the HPLC-PDA-MS equipment.

2.6. Chromatographic measurements

ESI was used for the analysis of weld and old fustic and APCI was used for the analysis of samples dyed with madder and cochineal. Some among the colouring molecules associated to the considered dyeing materials (i.e.: apigenin and luteolin for weld; kaempferol for old fustic; carminic acid for cochineal; alizarin and purpurin for madder) were available in the laboratory as commercial products. These were dissolved in MeOH and used as references to determine retention times and test the response of the mass detector in positive and negative ionisation modes. In these cases,

the MS acquisition (high resolution mode) was performed with data-dependent tandem mass spectrometry. Direct infusion into both the ESI and the APCI ion sources in positive and negative ionisation modes of the standard molecules was used for the optimisation of MS acquisition. The optimised parameters, which were then used to recognise the target molecules in the chromatograms from the sample extracts, are reported in Table 2. All the extracts were injected three times. The chromatographic peaks obtained by PDA or MS detectors were integrated and mean and standard deviation were calculated for the triplicate injection. Relative standard deviation was less than 5% for MS analysis and about 3.5% for the PDA detector. Therefore, because of their higher precision, the PDA signals (peak areas) were used for calculating the percentage of the molecules that have not been degraded under the LED lamps. Evaluation of the repeatability of the overall extraction procedure was performed by extracting in parallel three samples taken from unexposed silk fabrics and by considering the peak areas from the PDA detector. The relative standard deviation resulted lower than 20% for all the considered molecules, therefore this figure was adopted to calculate the precision of the results. After having checked the linearity of the signal/concentration relation, the percentage of each dye remaining after the fading experiment was calculated comparing the chromatographic peak areas (normalised on the exact weight of the textile sample) before and after the fading experiment.

3. Results and discussion

3.1. Spectral and colourimetric data

Table 3 reports for all samples the CIE L*a*b* and L*C*h* coordinates calculated using the equi-energy spectrum before and after the exposure to LED. The use of equi-energy spectrum allows to focus only on the colour variation of the material itself not influenced by the spectral distribution of the lighting source.

Table 3 also shows the colour differences at the end of the fading experiment calculated using the CIE L*a*b* ΔE and the CIEDE2000 formulas. It is to note that CIEDE2000 underestimates the colour differences for all samples, indeed [29] states that CIEDE2000 is a colour difference formula for small colour differences and the validity of the application of CIEDE2000 to CIELAB colour

differences greater than 5 ΔE is under investigation (a CIE Technical Committee, namely the TC 1-63 Validity of the Range of CIEDE2000, has been specifically appointed to clarify this point). Unfortunately a conclusive answer for a totally satisfactory colour difference formula is not available yet.

Figure 4shows the reflectance spectra of the samples at the beginning and at the end of the light exposure, and Figures 5, 6 and 7 show some examples of the chromatograms that were obtained from the dyed silk samples before the fading experiment; Figure 8 reports the percentage of the molecules which have been detected after the exposure to LED lighting in relation to the initial situation.

3.2. Flavonoids

The values of ΔE for samples containing flavonoids (Table 3) are significant of the decay suffered by dyestuffs with different light-fastness aptitude. By referring to the plants of origin, the light-fastness can be summarised as: weld > old fustic > brazilwood > logwood. This different aptitude is particularly represented by the variation on L* value attesting the changes of these samples to a paler colour; this variation is quite limited in the samples dyed with weld which, however, shows a higher decrease in terms of saturation (C*) when compared to the other samples.

With the only exception of samples dyed with old fustic, the hue (h*) of these samples also varies, particularly for samples dyed with logwood, which show the highest change in h* value. More in general, the well-known poor light-fastness of brazilwood and logwood was confirmed by the colourimetric data (Table 3).

Luteolin and apigenin were monitored with HPLC-PDA-MS (Figure 5) in samples dyed with weld. The chromatographic data (Figure 8) show the decline of the main colouring chemical species. Luteolin decreases to below 30% in samples exposed to NW-LED and WW-LED2, while it remains at about 47% after exposure to WW-LED1. Apigenin shows a similar trend, although with higher percentages, particularly if WW-LED1 is considered.

Morin was detected in the extracts of the sample dyed with old fustic (Figure 6) as the main colouring molecule. Two small peaks (at Rt. 13.23 and Rt. 16.08) showed molecular ions at *m/z* 287.056 $[M+H]^+$ and at 287.055 $[M+H]^+$ respectively and with MS^2 spectra having similar fragments, corresponding to the fragmentation pattern of kaempferol, a minor coloured component in old fustic. Morin and kaempferol showed a marked decay after the exposure to the three LEDs. In the samples exposed to NW-LED and to WW-LED2, morin decreased below 5% and 1% respectively, while remained at 10% of its initial value after exposure to WW-LED1. The decrease of kaempferol isomers (calculated as sum of the chromatographic areas) is smaller than that of morin, with percentage values of about 12%, 37% and 27% for samples exposed to NW-LED, WW-LED2 and WW-LED 1 respectively, as shown in Figure 8.

The lower light-fastness of old fustic found with respect to weld may be attributed to the structure of flavonols, with an extra hydroxyl in the C3 position. As reported in the literature [30], the degradation pathway can occur with an initial photo-oxidation on the double bond C2–C3 promoted by the action of a radical moiety. The higher electron density of the C2–C3 double bond in flavonols can promote the initial oxygenation which is followed by the breaking of the C2–C3 and C3–C4 bonds. Thus, if oxygen is involved as an intermediate in the photo-oxidation of flavonoids, this high reactivity with singlet oxygen would contribute to the lower photo-stability of the flavonols morin and kaempferol, compared to the flavones luteolin and apigenin.

Considering the different influences that the three different LEDs may have had on the samples, Table 3 indicates that no significant difference emerge when the colourimetric measurements are considered, whereas HPLC-PDA-MS data, reported in Figure 8, show instead that the sample response is different if each single dyeing molecule is considered.

In particular WW-LED1, which has a lower colour temperature and then a lower emission in the blue region, has definitely less influence on the yellow molecules such as luteolin, apigenin (from weld), and morin and kaempferol (from old fustic).

3.3. Anthraquinones

The samples dyed with madder and cochineal show a lower colour change when compared with samples dyed with flavonoids, in agreement with their structural characteristics. For these samples, ΔE and variation in hue and L* follow a similar trend. After exposure to the three LED lamps, a small increment in the C* value can be observed in samples dyed with cochineal. The ΔE data normalised on the effective light dose emitted by the different LEDs (ΔE /light dose ratio) at the end of exposition period (shown in Table 3), confirm the trend described for the decay.

As for samples dyed with cochineal, the main colourant compound (carminic acid) was easily detected in the HPLC-PDA-MS runs, together with other several red compounds. These are present at a barely detectable level and they could not be attributed to known compounds pertinent to cochineal.

By comparing the chromatographic profiles of the samples before and after exposure to the different LEDs, relevant modifications of the chromatographic signals emerge, as shown in Figure 9. In particular, a shoulder appears in the peak at 11.57 min and a significant decrease of the same peak occurs in the chromatogram of the sample exposed to WW-LED2. The peak at 12.05 min, just hinted in the chromatogram of the initial sample, greatly increases after the LED lights illumination and a shoulder at 12.35 min appears in the chromatograms after the exposure to WW-LED1 and NW-LED. In addition, a new peak at 13.74 min is detected in the chromatograms of all the samples exposed to the LEDs.

The contribution of these minor red compounds was calculated as the sum of the areas of their chromatographic peaks, normalised with respect to initial carminic acid (Figure 10). In the sample not exposed to light, the sum of these minor red compounds is *ca.* 8% of the carminic acid amount and it increases to 30% and 50% after illumination with NW-LED and WW-LED1, respectively. On the contrary, it remains at the initial level (about 9%) after illumination with WW-LED 2. The peak of carminic acid decreases to 62% in samples exposed to NW-LED and to 46% and 50% after exposition to WW-LED2 and WW-LED1, respectively.

HPLC analyses therefore revealed that the photo-degradation of carminic acid is accompanied by the formation (or the increment) of other red compounds, and this is an interesting topic that deserves further investigation aimed at characterising the compounds and, possibly, linking them to the photo-degradation pathways of carminic acid.

In the samples dyed with madder, alizarin, purpurin and munjistin were monitored (Figure 7). Alizarin, the main colouring molecule in madder, decreases at 58% after exposition to NW-LED and at 85% and 60% when exposed to WW-LED1 and WW-LED2 (Figure 8).

The influence of the LEDs was stronger for purpurin. It was found at about 40% and 50% of the initial concentration in samples exposed to NW-LED and WW-LED1, and even at about 30% in the sample exposed to WW-LED2. Munjistin dropped at 18%, 45% and 31% of the initial concentration for the NW-LED, WW-LED2 and WW-LED1 respectively.

The differences between the degradation occurring in alizarin, munjistin and purpurin are related to their molecular structure; in agreement with the literature data. It is confirmed here that the light-fastness of anthraquinones decreases as the number of hydroxyl substituents increases [4].

As already evidenced for the flavonoids, WW-LED1 appears to have less influence on purpurin than the other two LEDs, which have a major emission peak centred at 480 nm, where purpurin as a maximum of absorption.

4. Conclusions

The overall results indicate that the high light dose employed in the experiments has caused detectable colour fading in the tested samples.

It is to note that the samples were exposed to a light dose uncommon in real museum light environments compliant with [11] and [14] suggestions and requirements, but such an unfavourable condition was functional to the scope of the research, which is the evaluation of the damage potential of the three LED lamps with different CCT. The study enlarges the knowledge of the damage that can be induced by different LED spectra, and provides information about museum

lighting source selection, that should not be based only on Colour Rendering Index (CRI) capabilities, but also on the damage potential of the spectral distribution of the source.

By considering the colour temperature of the different white LEDs and their related emission spectra, it emerges that WW-LED1 causes the lowest photo-degradation on molecules which absorb in the blue region of the spectrum. On the contrary, NW-LED, which has a higher energy emission in the range from 400-500 nm has a more extensive effect.

Considering the ΔE values at the end of the exposition to NW-LED (the worst case) samples coloured by molecules belonging to the anthraquinones (madder and cochineal) showed the lowest differences (lower than 10 ΔE), while brazilwood, logwood and old fustic showed the largest ones (larger than 20 ΔE. These results are in partial conflict with [16] were cochineal and madder are indicated as high responsivity materials. Nevertheless, the chromatographic data support this indication, as significant changes in the initial concentration of the colouring species were detected. Moreover, the results also give general information of the two approaches that were employed to investigate the photo-degradation of the dyes under the LEDs, i.e.: colourimetry and chromatography. Colourimetric measurements, widely adopted for fading evaluation, can provide a fast and general information on the variations of colour, whereas HPLC-PDA-MS technique has demonstrated to have higher sensibility for monitoring the fading process. The chromatographic insight enables in fact a more precise distinction between the effects of the different LEDs lamps on the considered textiles. Moreover, the invasive approach by HPLC-DAD-MS was able to highlight that the small differences in colour, which were detected by the non-invasive colourimetric measurements, are actually associated with relevant variations in the original concentration of the colouring species. This result offers a more in-depth insight into the actual damage potential of LED lighting on natural dyes.

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Captions to figures

Fig. 1 Spectral irradiance distribution of the three investigated white LED lamps.

Fig. 2 Structures of the dyes discussed in the text
a) Apigenin: R2=OH. Luteolin: R2=OH; R3=OH. Morin: R1=OH; R2=OH; R4=OH. Kaempferol: R2=OH; R4=OH.
b) carminic acid (Natural Red 4)
c) Purpurin: R=OH; Alizarin (Mordant Red 11): R=H.
d) Mujistin
e) Brazilin: R=H; Haematoxylin (Natural Black 1): R=OH
f) Brazilein: R=H; Haematein: R=OH

Fig. 3 Reflectance spectra of silk samples before exposure to LEDs illumination.

Fig. 4 Chromatograms for a sample dyed with weld
A difference in the retention time (about 0.1-0.2 min) is expected between PDA and MS chromatograms, as the detectors are arranged in series.
Black line: PDA chromatogram (250-350 nm): Rt 16.57 = luteolin; Rt 18.67 = apigenin
Red line: MS chromatogram of luteolin (selected ion $[M+H]^+$ = 287.055)
Green line MS chromatogram of apigenin (selected ion $(M+H)^+$ = 271.061

Fig. 5 Chromatogram of a sample dyed with old fustic
A difference in the retention time (about 0.1-0.2 min) is expected between PDA and MS chromatograms, as the detectors are arranged in series.
Black line: PDA chromatogram (250-350 nm): Rt 12.58 = morin; Rt 13.23 = kaempferol isomer; Rt 16.08 = kaempferol isomer
Red line: MS chromatogram of kaempferol (selected ion $(M+H)^+$ = 287.056.
Green line: MS chromatogram of morin (selected ion $(M+H)^+$ = 303.051

Fig. 6 PDA chromatogram (400-500 nm) of sample dyed with a) cochineal: Rt 10.85 = carminic acid and b) madder: Rt 22.27 = alizarin; Rt 24.74 = purpurin; Rt 27.77 = munjistin.

Fig. 7 Residual percentage of marker molecules monitored by HPLC-PDA-MS analysis.

Fig. 8 Expanded PDA chromatograms (400-500 nm) of cochineal samples, where modifications of peaks at Rt. 11.57, 12.05 and 13.75 min are highlighted.

Fig. 9 Percentage of red colourants in samples dyed with cochineal, obtained on HPLC data by considering 100% the area of the chromatographic peak of carminic acid before the fading experiment.

Figure 1

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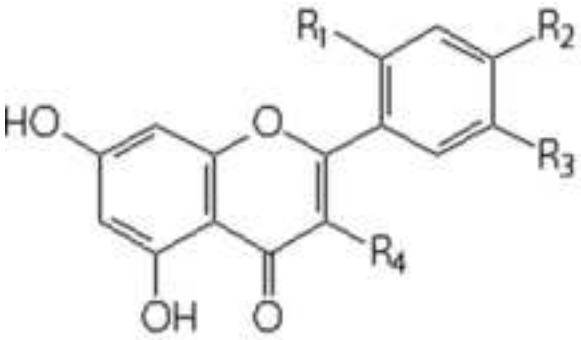

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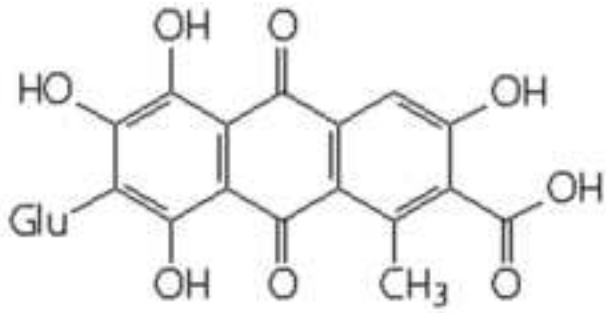

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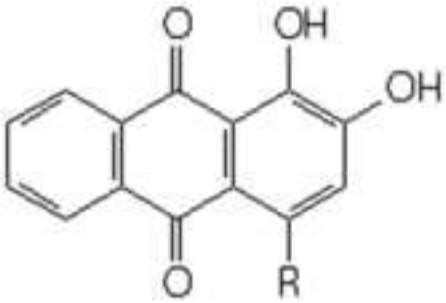

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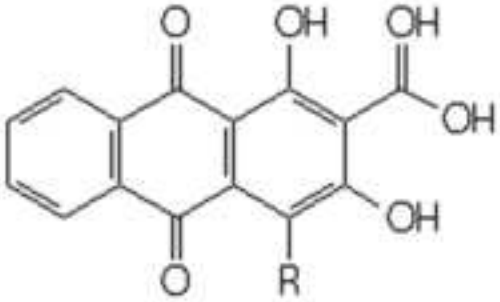

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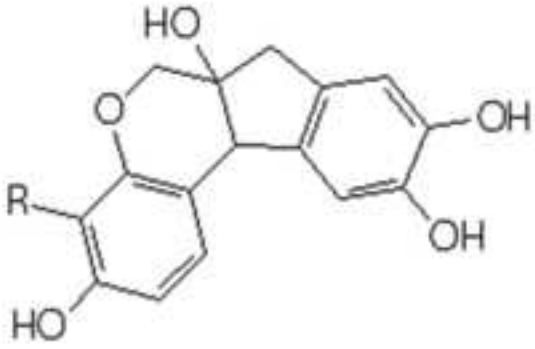

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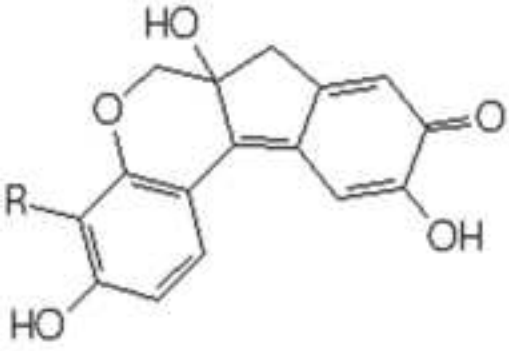

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Figure 2

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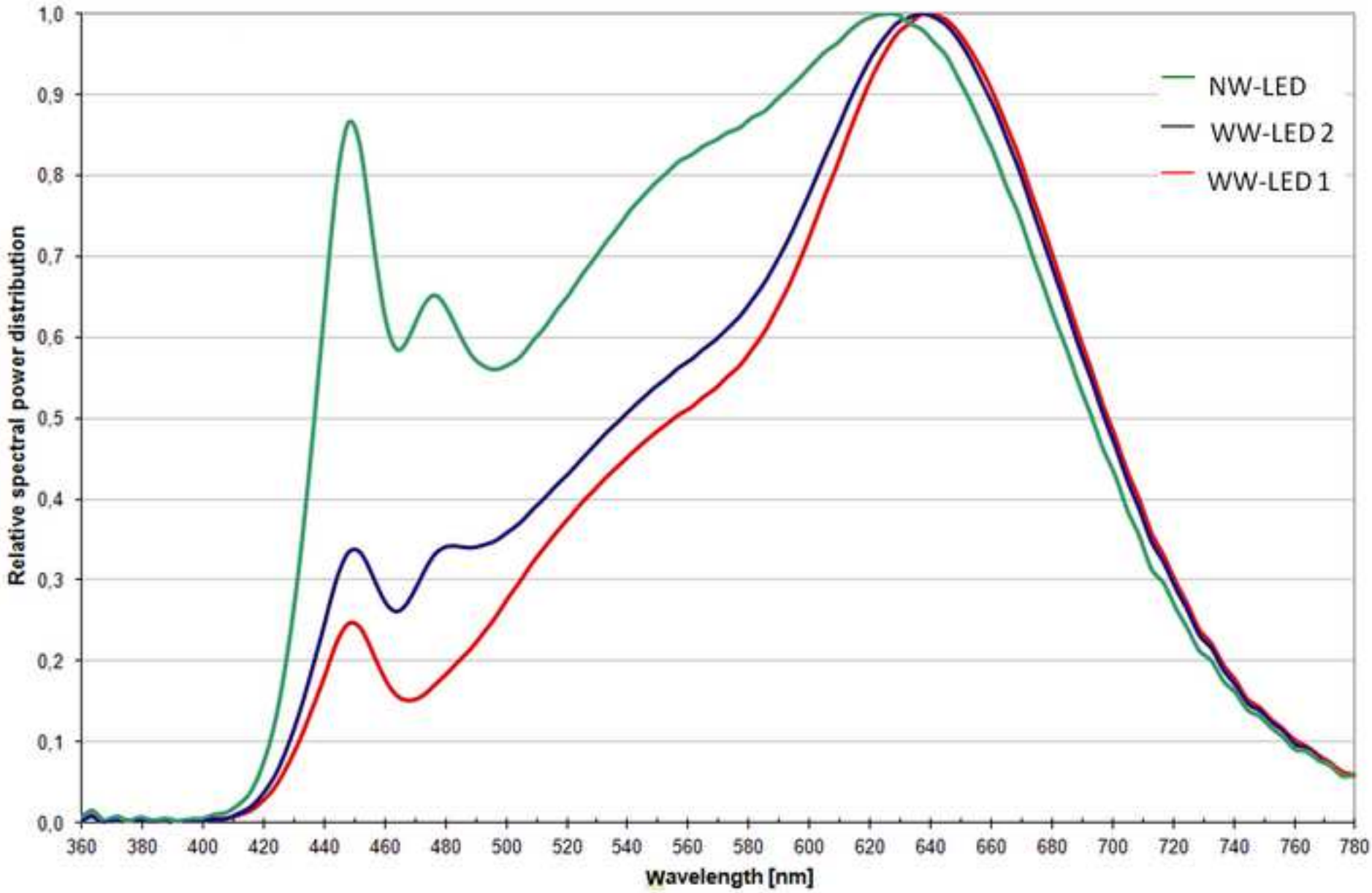

Figure 3

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Figure 4

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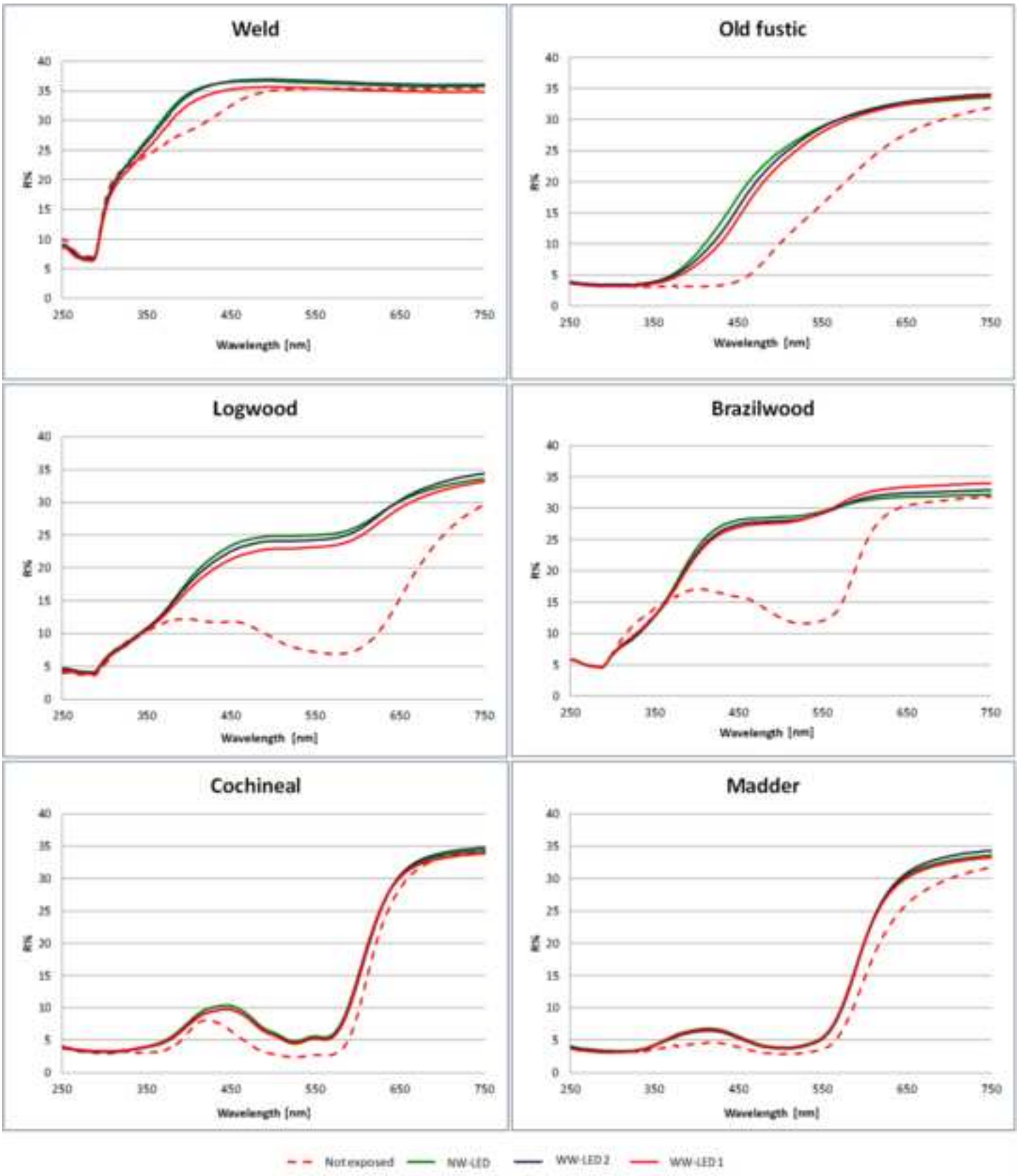

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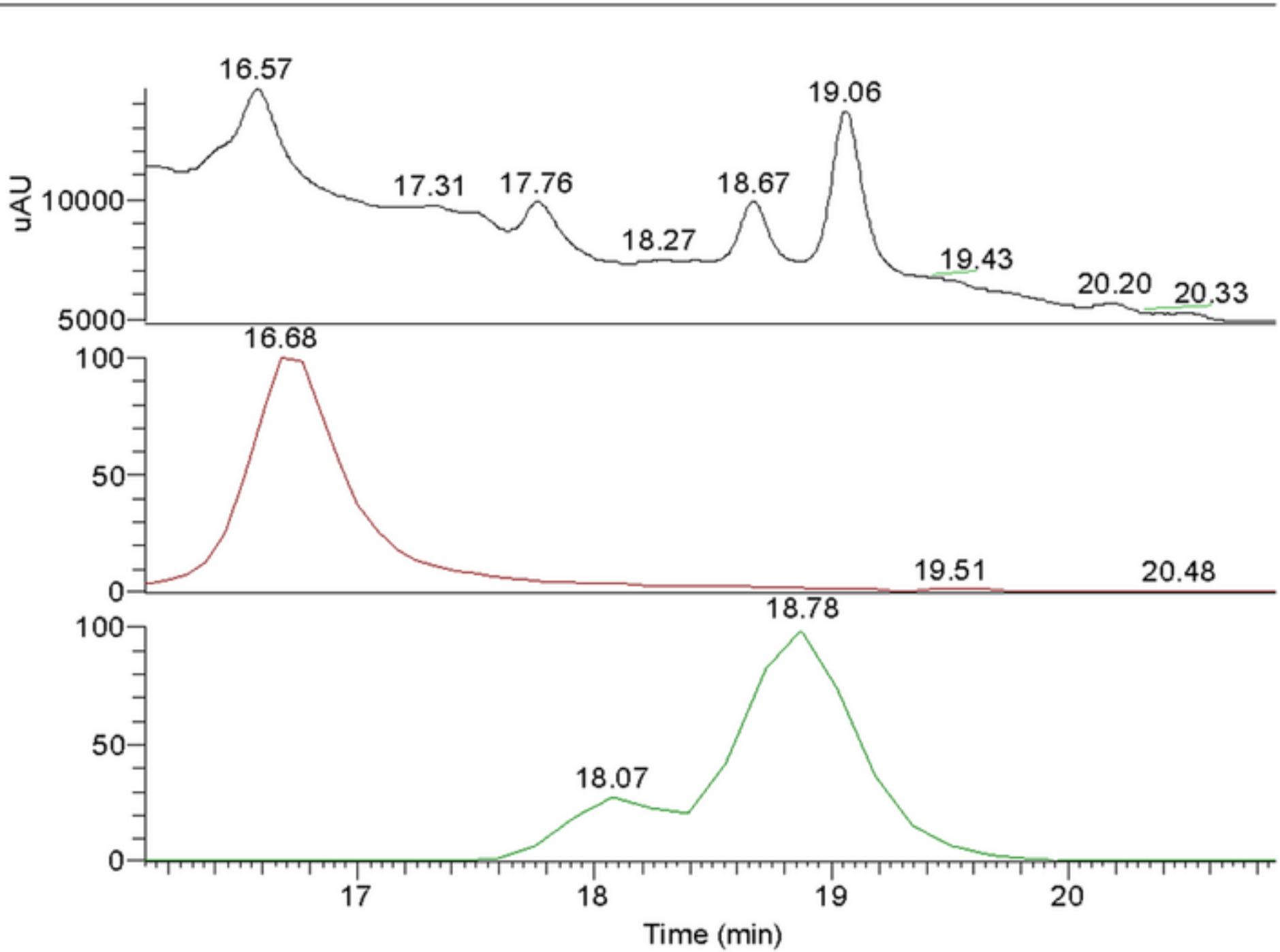

Figure 6

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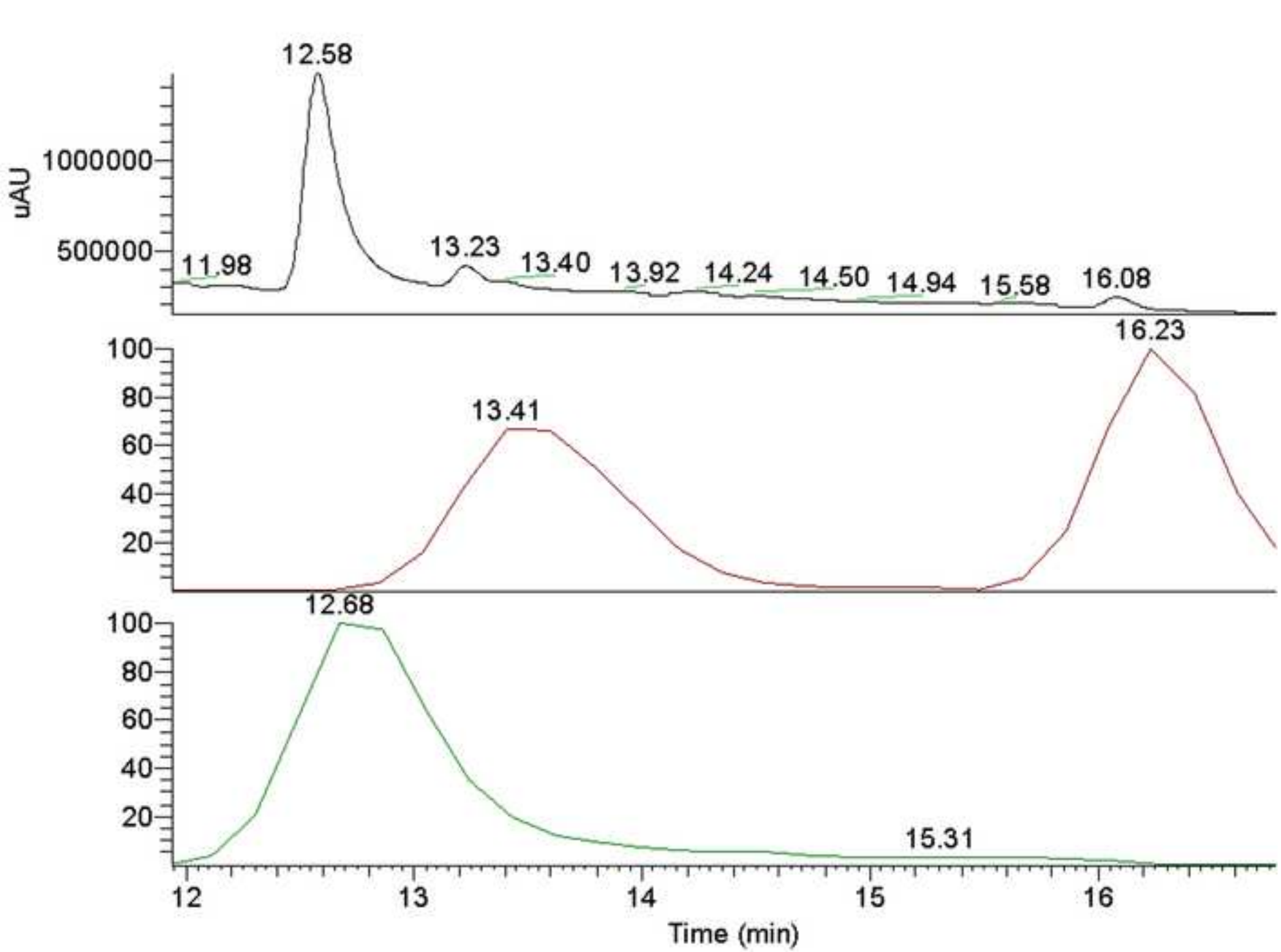

Figure 7a

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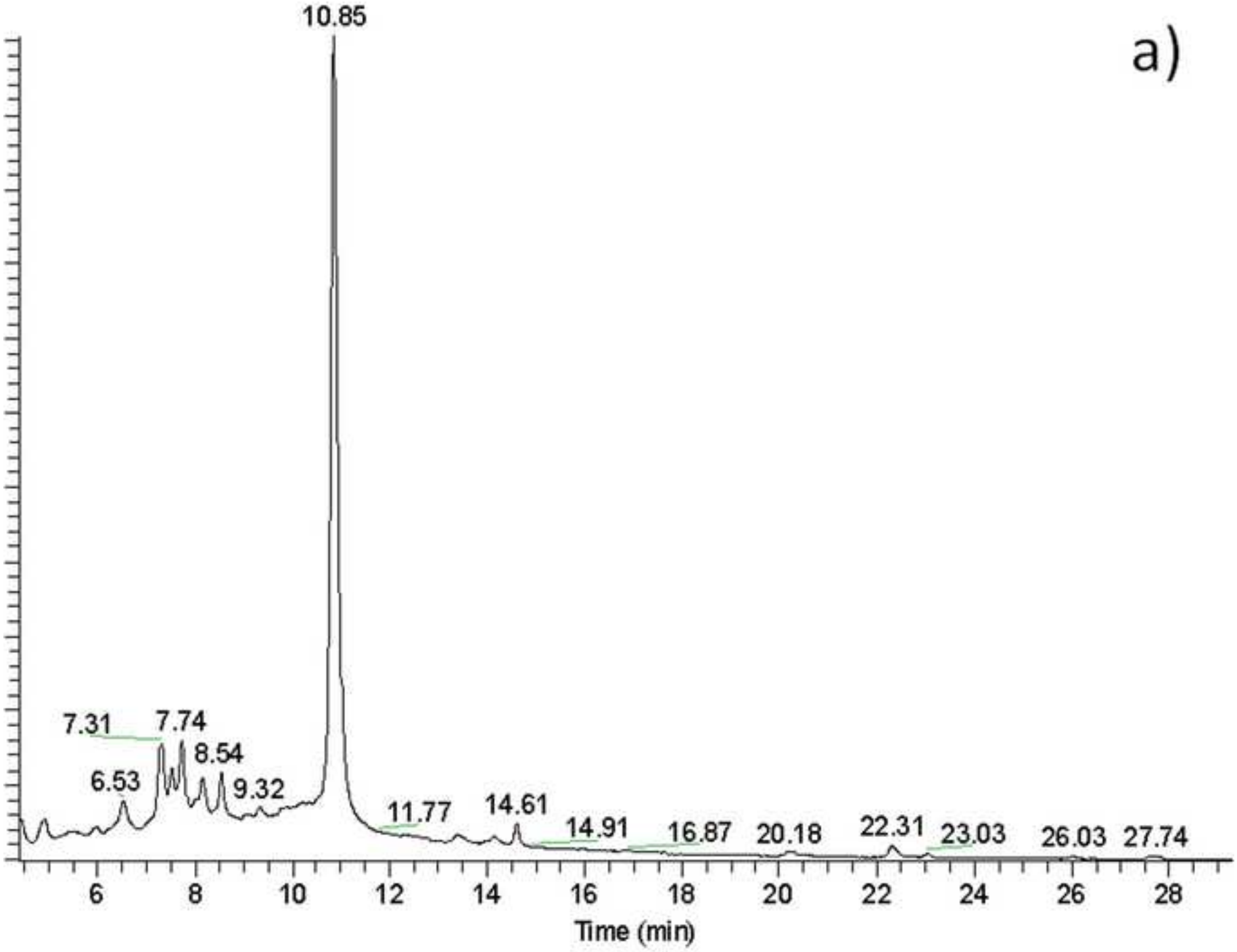

Figure 7b

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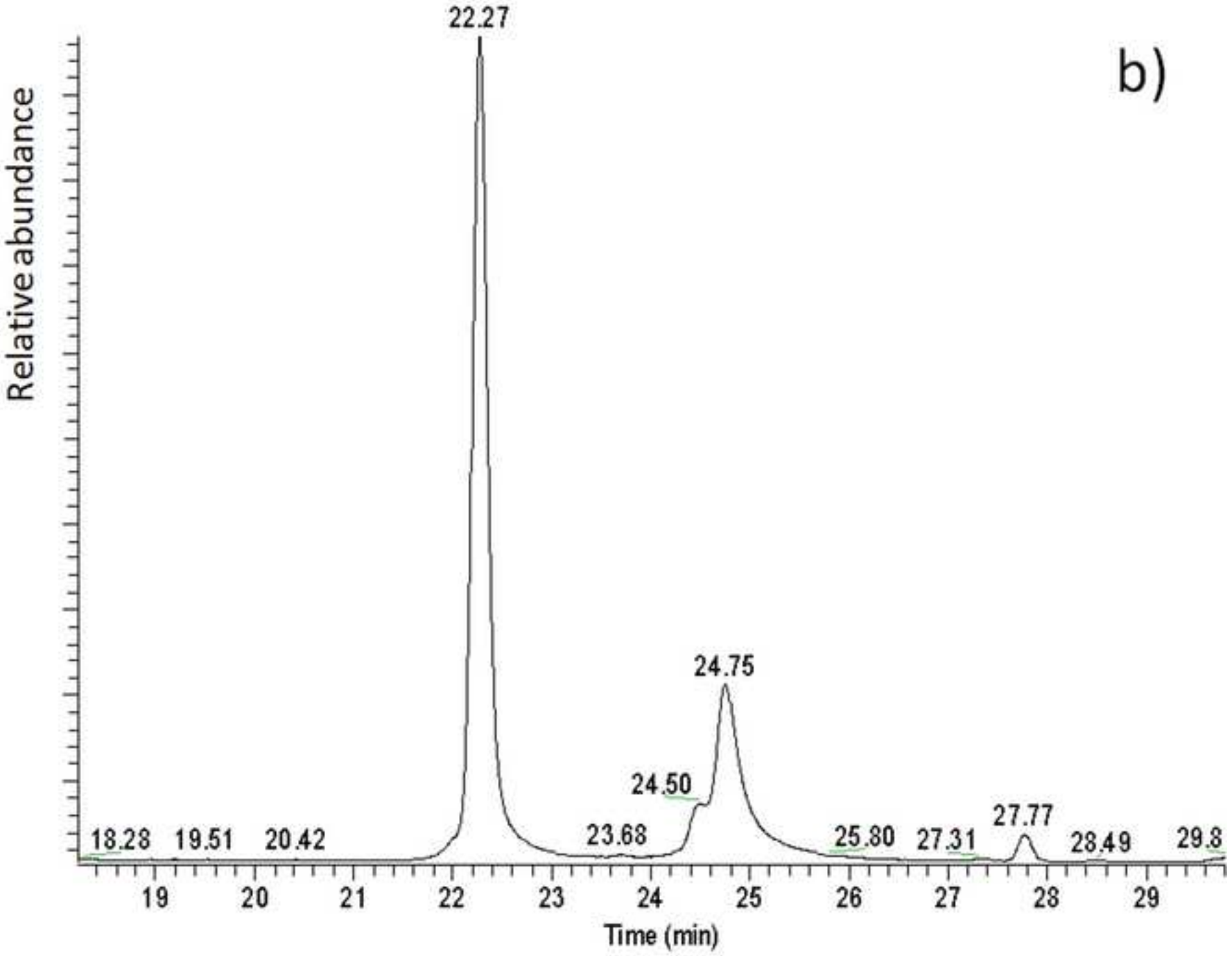

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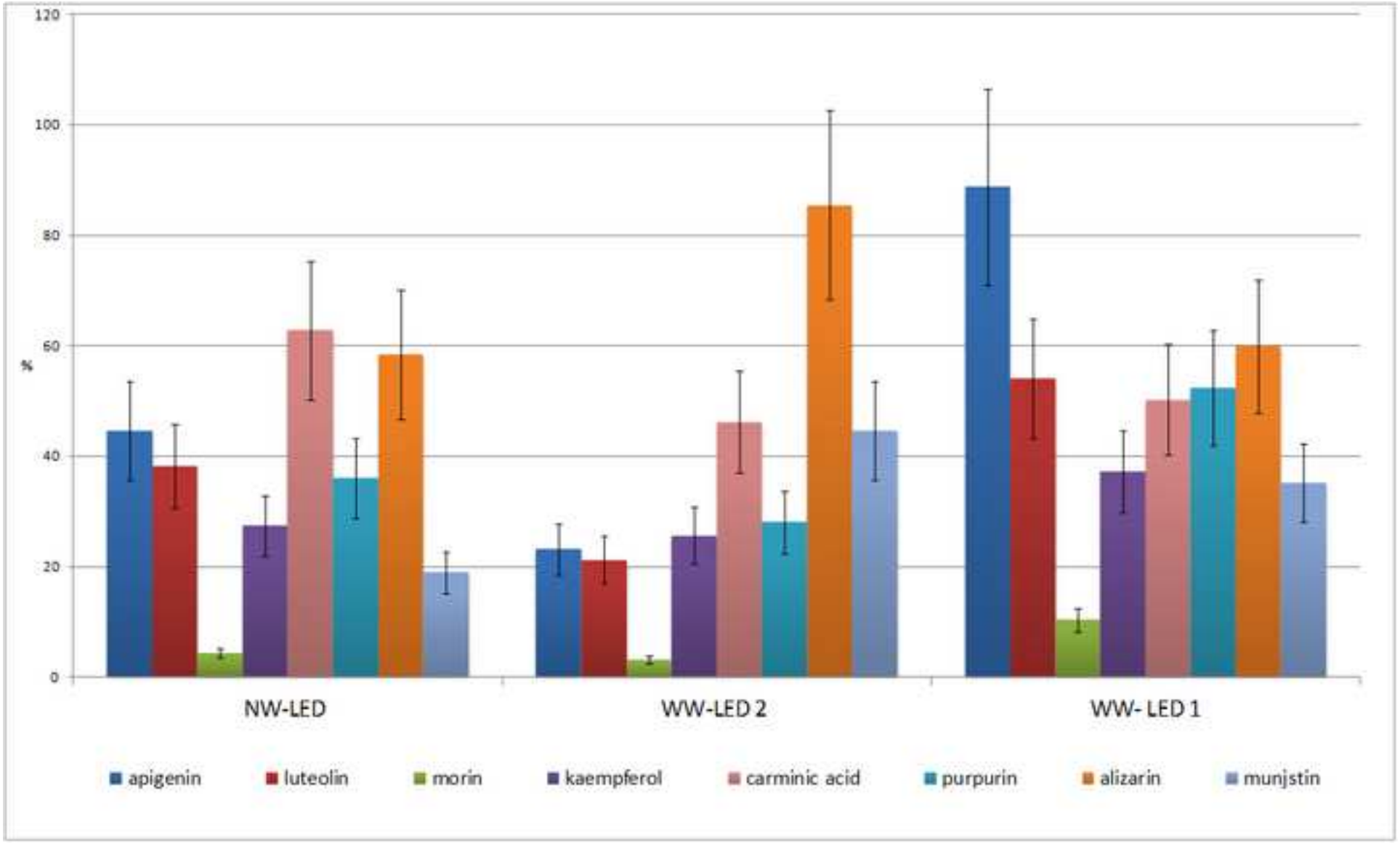

Figure 9

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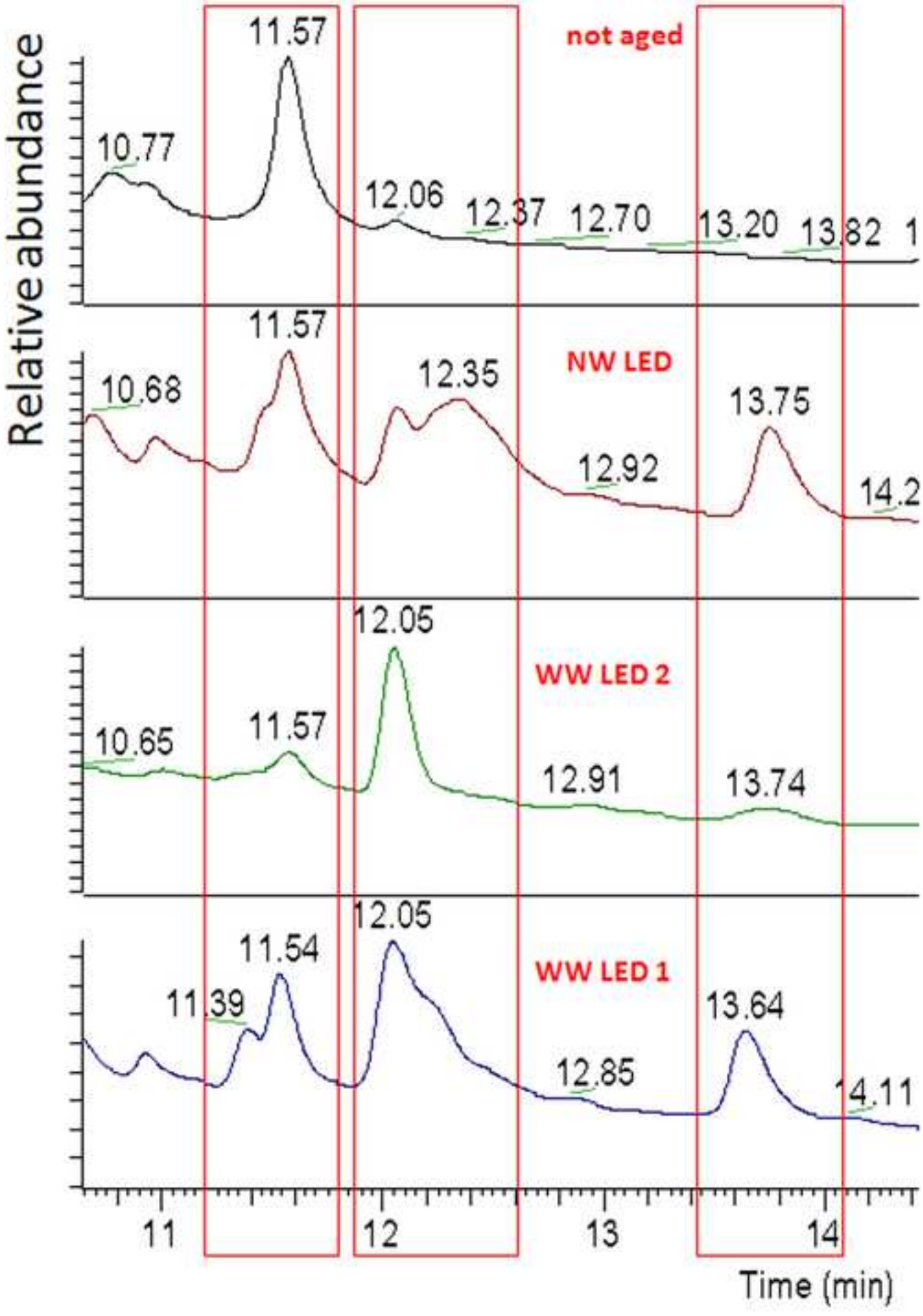

Figure 10

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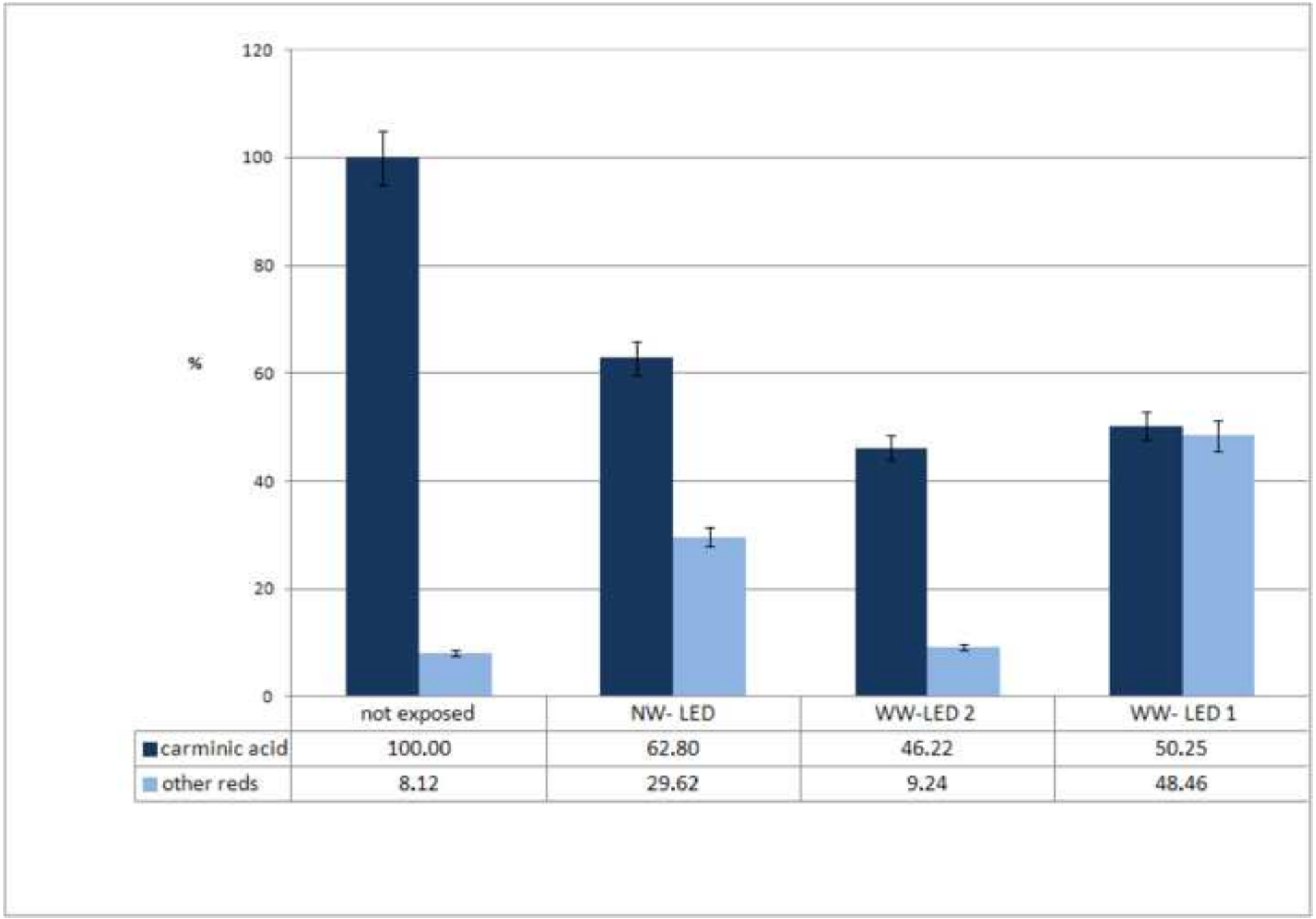

	not exposed	NW- LED	WW-LED 2	WW- LED 1
carminic acid	100.00	62.80	46.22	50.25
other reds	8.12	29.62	9.24	48.46

Table 1: Photo-radiometric characteristics of the investigate LED sources

LED type	Correlated Colour Temperature [K]	UV-A ratio [µW/lm]	Vis-NIR ratio [mW/lm]	Luminous flux [lm]
NW-LED	3892	0.43	2.87	700
WW-LED2	2853	0.57	3.15	700
WW-LED1	2581	0.58	3.10	700

Table 2

Table 2: Spectrometric characteristics of the molecules detected in HPLC-DAD-MS analysis.

Dye	Compound	Mass Weight	Ionization source	$[M+H]^+$ (m/z)	MS^2 Characteristic fragment ions (m/z)	Absorbance maxima (nm)
Weld	Luteolin	286	ESI+	287.055	287.056; 153.018	208; 254; 266; 348
	Apigenin	270	ESI+	271.061	271.061; 153.019	268; 336
Old Fustic	Morin	302	ESI+	303.051		248; 295; 345
	Kaempferol	286	ESI+	287.056		266; 366
Cochineal	Carminic acid	492	APCI+	493.105		226; 268; 313; 486
Madder	Purpurin	256	$APCI^+$	257.045	239.034; 229.050; 187.039	256; 292; 481
	Alizarin	240	$APCI^+$	241.050	223.080; 213.055; 137.079	200; 249; 278; 428
	Munjistin	284	APCI+	285.048		246; 277; 412

Table 3

Table 3. Colorimetric data on silk samples before and after the fading experiment.

Dye	LED lamp	Before					After					ΔE	CIEDE2000
		L	a*	b*	C	h	L	a*	b*	C	h		
Weld	NW-LED	62.9	7.0	16.2	17.6	66.6	66.7	5.6	3.8	6.8	34.2	13.1	9.5
	WW-LED2	63.3	7.0	16.9	18.3	67.5	67.0	5.5	4.1	6.9	36.7	13.4	9.6
	WW-LED1	62.8	7.1	17.2	18.6	67.6	66.1	5.4	4.3	6.9	38.5	13.4	9.6
Old fustic	NW-LED	49.1	12.5	40.7	42.6	72.9	60.7	4.1	22.5	22.9	79.7	23.1	13.9
	WW-LED2	49.1	12.9	40.3	42.3	72.3	60.5	4.2	25.6	25.9	80.7	20.6	13.2
	WW-LED1	49.7	12.3	41.4	43.2	73.5	59.9	4.3	27.7	28.0	81.2	18.8	11.9
Logwood	NW-LED	34.3	13.7	-7.6	15.7	331.0	57.7	7.3	7.5	10.5	45.8	28.5	25.8
	WW-LED2	35.3	13.8	-7.3	15.6	332.1	57.1	7.8	7.9	11.1	45.4	27.2	24.5
	WW-LED1	35.4	13.6	-7.4	15.5	331.4	55.9	7.8	8.1	11.2	46.1	26.4	23.5
Brazilwood	NW-LED	47.5	27.5	5.2	28.0	10.7	61.6	7.3	6.8	10.0	43.0	24.7	19.4
	WW-LED2	47.8	27.9	5.2	28.4	10.6	61.5	8.1	7.7	11.2	43.6	24.2	18.9
	WW-LED1	47.9	28.4	5.1	28.9	10.2	61.7	8.8	8.7	12.4	44.7	24.3	18.9
Cochineal	NW-LED	29.5	44.3	2.5	44.4	3.2	36.7	38.3	2.8	38.4	4.2	9.4	6.2
	WW-LED2	29.1	43.7	2.8	43.8	3.7	36.1	38.7	3.3	38.8	4.9	8.6	5.9
	WW-LED1	29.5	43.9	2.7	44.0	3.5	36.1	39.1	3.4	39.2	5.0	8.2	5.5
Madder	NW-LED	33.9	37.9	20.3	43.0	28.2	39.6	38.5	22.0	44.3	29.7	6.0	4.9
	WW-LED2	34.2	38.6	20.9	43.9	28.4	39.2	39.3	22.7	45.4	30.0	5.3	4.3
	WW-LED1	34.3	38.1	20.4	43.2	28.17	39.3	38.4	22.1	44.3	29.9	5.3	4.3