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Abstract

The exhibition in museums of archaeological and historical textiles must keep into consideration the possible photo-degradation of the dyes. In the last decade, Light Emitting Diodes (LEDs) have been extensively replacing other light sources; nevertheless a 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 by considering the actual damage potential of LEDs on natural dyes, which is relevant for selecting the most suitable 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. Lighting shall 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], and the chemical structure of the molecules that are responsible for the colour is an important intrinsic factor that influences light-fastness. Both the skeleton structures of the various chemical families of dyes and the position of auxochromic 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 their negligible UV and infrared components in the 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 historical textiles dyed with natural dyes are available [10, 11].

This work aims at evaluating the suitability of LED lighting for illuminating historical textiles in display cases. Both the colour changes (i.e. the variation of colour coordinates) and the modifications that occurred in the concentration of the various colouring molecules were determined on silk cloths dyed in the laboratory.

Samples were obtained by dyeing silk clothes with plants (or insects) selected among the materials that have been most widely used in the past for dyeing [12, 13]. Two chemical families of dyes were considered: flavonoids (from weld, old fustic, logwood and brazilwood) and anthraquinones

(from cochineal and madder). The samples therefore represent 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 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 as requested by the Italian Cultural Heritage Preservation Act (50 klx h/year light dose) [14]; such a high light dose was chosen for our experiments in order to amplify the fading, with the aim of enhancing the possibility of recording detectable differences among the three lamps when measuring the colour coordinates and of obtaining significant colour variations that would enable 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 photo-diode array and mass spectrometric detectors (HPLC-PDA-MS) was used to this aim. The dyes extracted from the fabrics where analysed 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 LED light were collected by an UV-Vis-NIR Perkin Elmer Lambda 900 double-beam spectrophotometer measuring the spectral reflectance of each sample as follows: 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 with a PDA detector and a LTQ-Orbitrap analyser (Thermo Scientific) 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 by Phenomenex) 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 material 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 dye-bath, and heated at 90 °C for 30 min. After cooling, the dyed silk clothes were recovered from the baths, rinsed with water and dried at room temperature. They were then stored 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 each inside one dedicated fading box, designed and built specifically for this experiment. Each of the three boxes 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] suggests 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 by 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 throughout the 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.

In order to reduce repositioning errors, the samples were centred with respect to the entrance aperture of the spectrophotometer's integrating sphere using an alignment frame specifically prepared for each sample. Before each session of measurements, the following procedure was followed for spectral reflectance: 20 minutes instrument warm-up, internal calibration and grating alignment, baseline acquisition, a reference Spectralon standard was employed for testing the instrumental response at the beginning and at the end of the measurement trial. Repeatability and

reproducibility of the instrument were taken into account in the evaluation of the uncertainty for spectral reflectance measurements.

During the collection of the data from one set, the other two were still exposed and the overall 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 according to a guide for uncertainty evaluation of the International Organisation for Standardisation (ISO) [18] and also taking into account the metrological characterisation of the spectrophotometer. The propagation of the uncertainty in evaluating ΔE is complex because of the inner correlations among X, Y and Z, 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 considering 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 from the textile fibres. 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 recovery of the colouring species. It is known that this method causes the cleavage of glycosidic bonds, nevertheless it enables the possibility of determining the percentage of 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. In order to ensure the same extraction conditions for all the samples, they all were treated in parallel. 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. The residues then were dissolved in 600 µl of H_2O/MeOH (1/1, v/v) and injected into the HPLC-PDA-MS equipment.

2.6. HPLC-PDA-MS analysis

ESI was used for the analysis of samples dyed with weld and old fustic and APCI was used for those 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 the 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 by the 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 LEDs. The use of equi-energy spectrum allows to focus only on the colour variation of the material itself, without any influence of the lighting source's spectral distribution.

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 worth noting that CIEDE2000 formula underestimates the colour differences for all samples, indeed [29] states that CIEDE2000 is more suitable for evaluating small colour differences and the validity of the application of CIEDE2000 to

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

Figure 4 shows 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 changes of L* values towards paler colour; the variation is quite limited in the sample dyed with weld which, however, shows a higher decrease of saturation (C*) when compared to the other samples.

With the only exception of samples dyed with old fustic, the hue (h*) also varies, particularly for the sample dyed with logwood, which shows the highest change in h* value. More in general, the well-known poor light-fastness of brazilwood and logwood is confirmed by the colourimetric data in 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 as the main colouring species in the extracts of the sample dyed with old fustic (Figure 6). Two small peaks (at Rt. 13.23 and Rt. 16.08) showed quasi-molecular ions at *m/z* 287.056 $[M+H]^+$ and at 287.055 $[M+H]^+$ respectively, with MS^2 spectra having similar fragments linked 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 to 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 the sum of the chromatographic areas of the two detected isomers) 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 (Figure 1). 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 cleavage 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 emerges when the colourimetric data 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 modest increment in the C* value is 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 the exposition - reported in Table 3 - confirm the trend described for the decay.

As for samples dyed with cochineal, the main colouring compound (i.e. carminic acid) was easily detected by HPLC-PDA-MS, together with some further red compounds. These are present at a barely detectable level and they could not be attributed to known compounds pertinent to cochineal.

As shown in Figure 9, By relevant modifications of the chromatographic signals emerge by comparing the chromatographic profiles of the samples before and after their exposure to the different LEDs. 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 fading experiment 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 peak area measured for 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 highlighted by HPLC-PDA-MS analysis for the stability of alizarin, munjistin and purpurin under LED lighting are related to their molecular structure: in agreement with the literature data [4], it is confirmed here that the light-fastness of anthraquinones decreases as the number of hydroxyl substituents increases (Figure 1).

As already evidenced for 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 our experiments has caused detectable colour fading in all 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 absorbing 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 general 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 are 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 non-invasive integrated information on colour variations, whereas HPLC-PDA-MS has demonstrated a 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.

Acknowledgements

The contribution of PRIN 2010-2011 project (Sustainability in cultural heritage: from diagnosis to the development of innovative systems for consolidation, cleaning and protection - Ministry of University and Research, Italy) is acknowledged for funding. Prof. Claudio Baiocchi is kindly acknowledged for making available the HPLC-PDA-MS equipment,.

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Captions to figures

Fig. 1 Structures of the molecules 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
c) Purpurin: R=OH; Alizarin: R=H.
d) Munjistin
e) Brazilin: R=H; Haematoxylin: R=OH
f) Brazilein: R=H; Haematein: R=OH

Fig. 2 Spectral irradiance distribution of the investigated LED lamps

Fig. 3 Image of the samples after the fading experiment (NW-LED) compared with the non-exposed ones. 1 = Weld; 2= Old Fustic; 3= Logwood; 4=Brazilwood; 5= Cochineal; 6= Madder

Fig. 4 Reflectance spectra of the silk samples before (dashed line) and after the fading experiments under the three different LED lamps

Fig. 5 Chromatograms of the 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 a 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. 6 Chromatogram of the 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 a 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. 7 PDA chromatogram (400-500 nm) of the samples dyed with a) cochineal: Rt 10.85 = carminic acid and b) madder: Rt 22.27 = alizarin; Rt 24.75 = purpurin; Rt 27.77 = munjistin.

Fig. 8 Residual percentage of marker molecules determined by HPLC-PDA-MS analyses.

Fig. 9 Expanded PDA chromatograms (400-500 nm) of cochineal samples, where modifications of the peaks at Rt. 11.57, 12.05 and 13.75 min are visible.

Fig. 10 Percentage of the minor red colourants in the sample dyed with cochineal. The percentages are calculated with respect to the peak area of the carminic acid at the beginning of the fading experiment.

Figure 1

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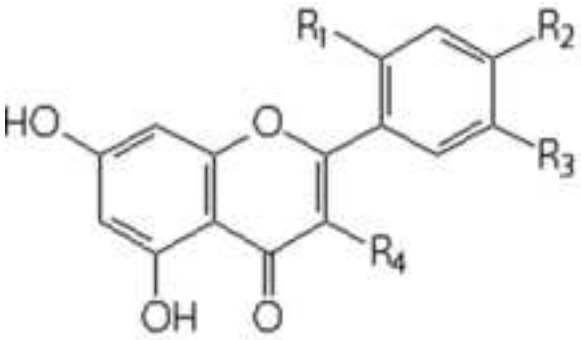

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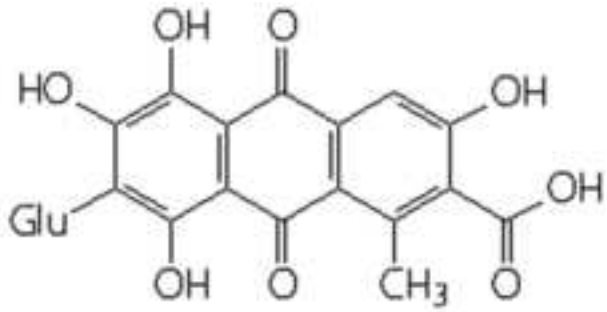

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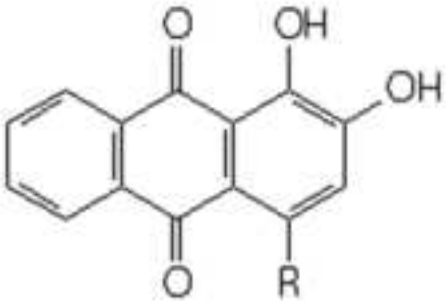

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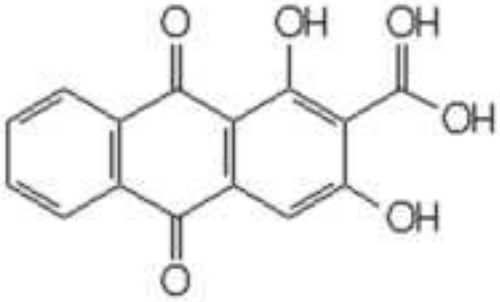

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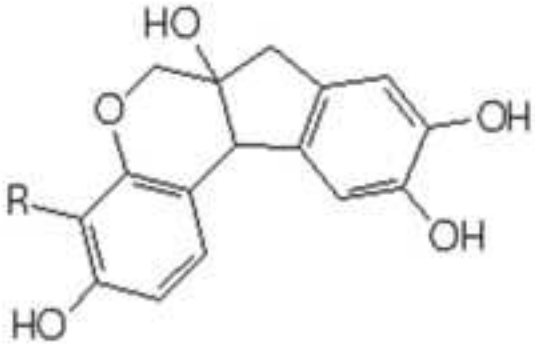

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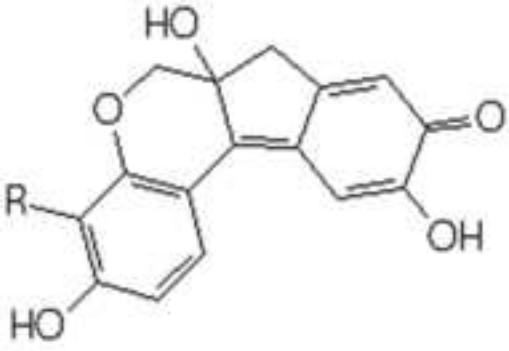

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Figure 2

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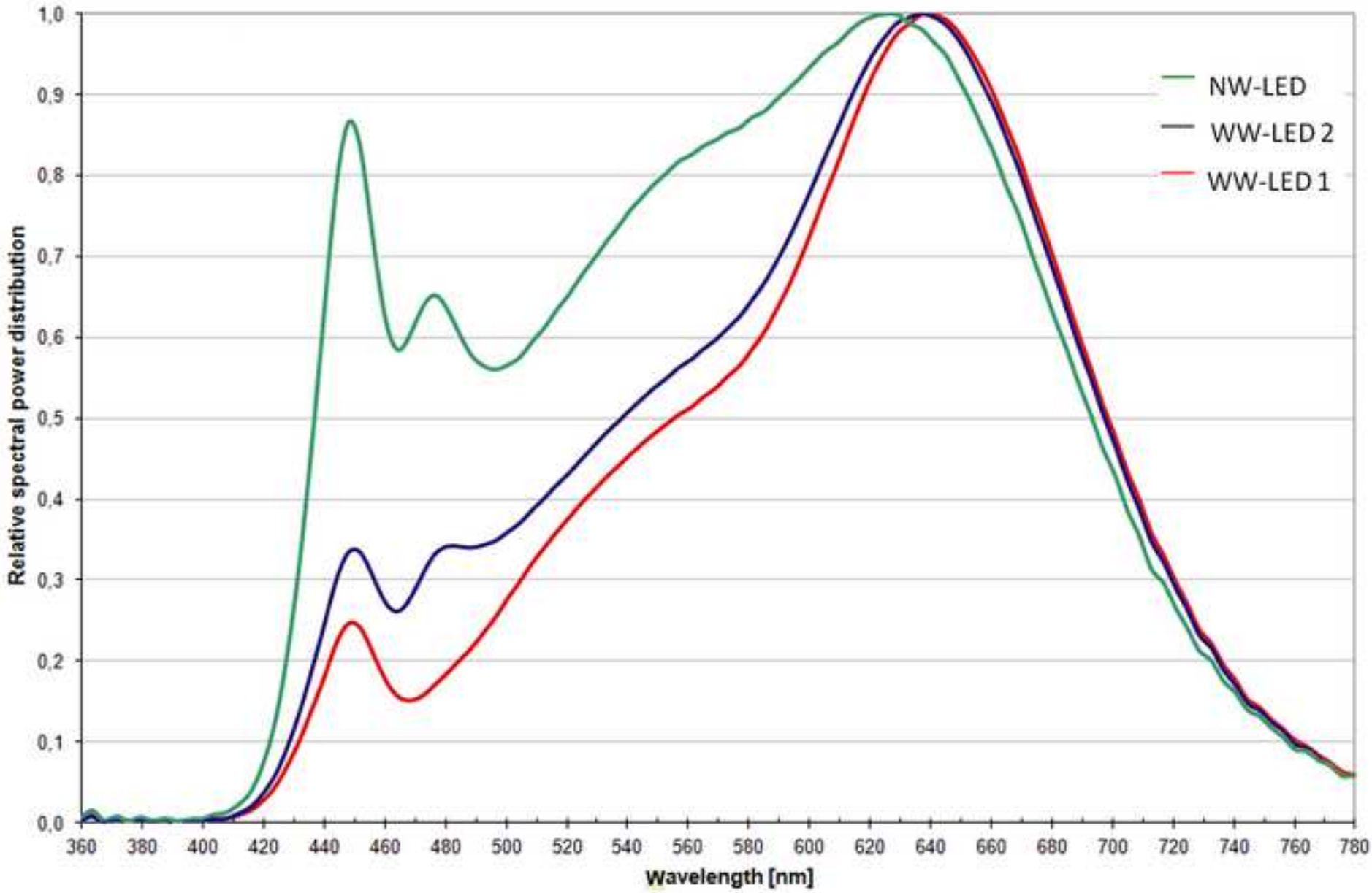

Figure 3

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Figure 4

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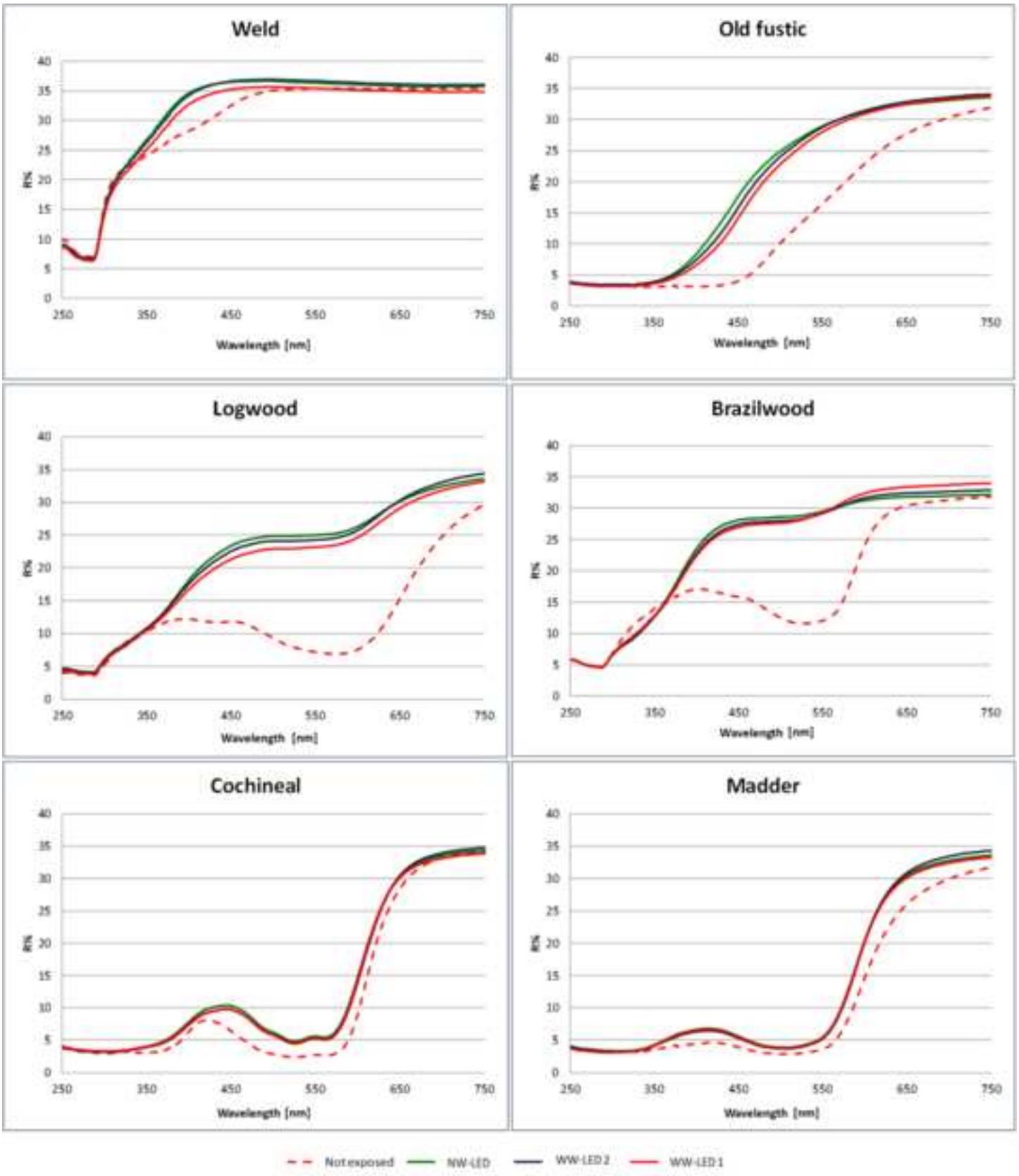

Figure 5

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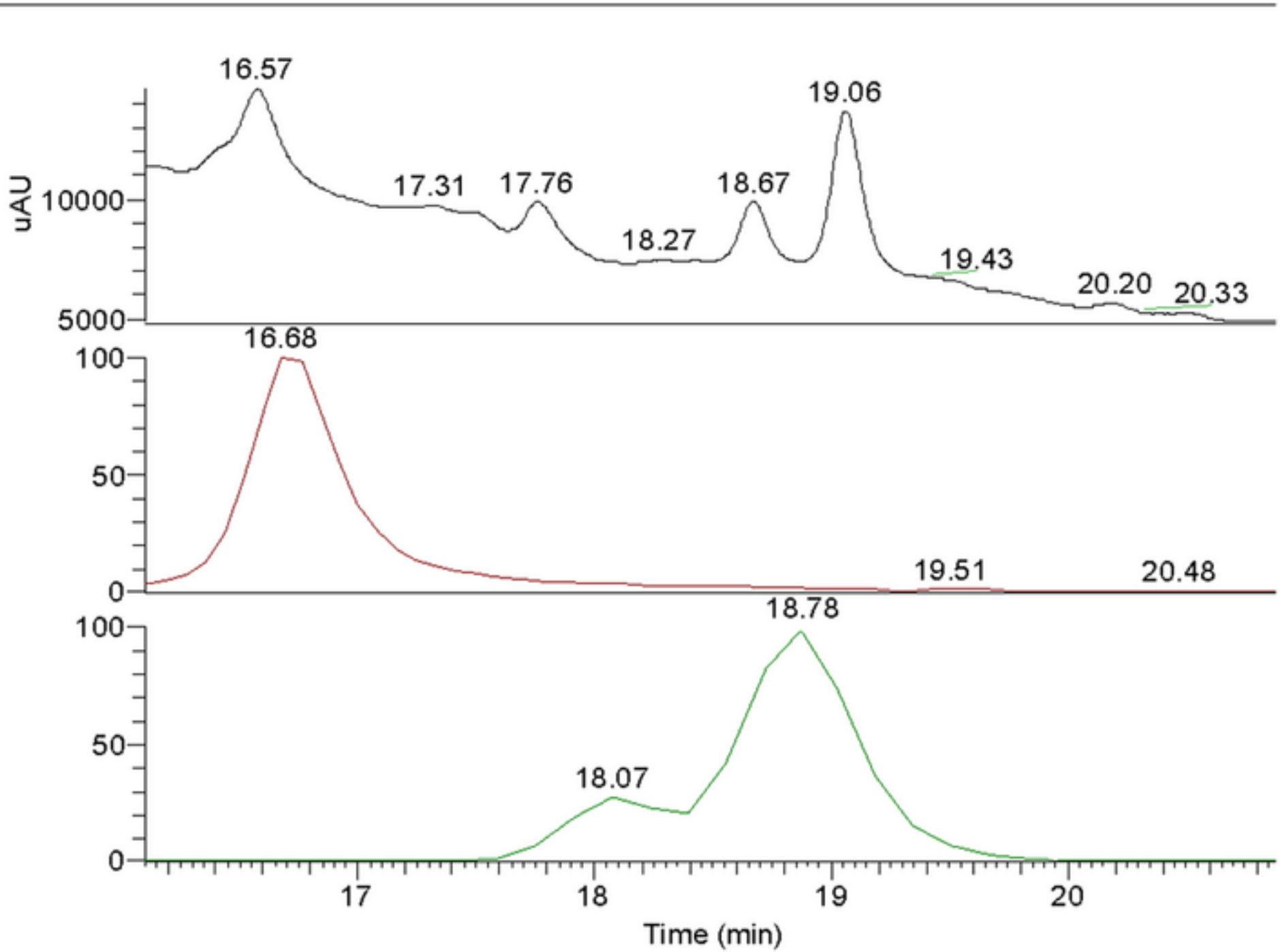

Figure 6

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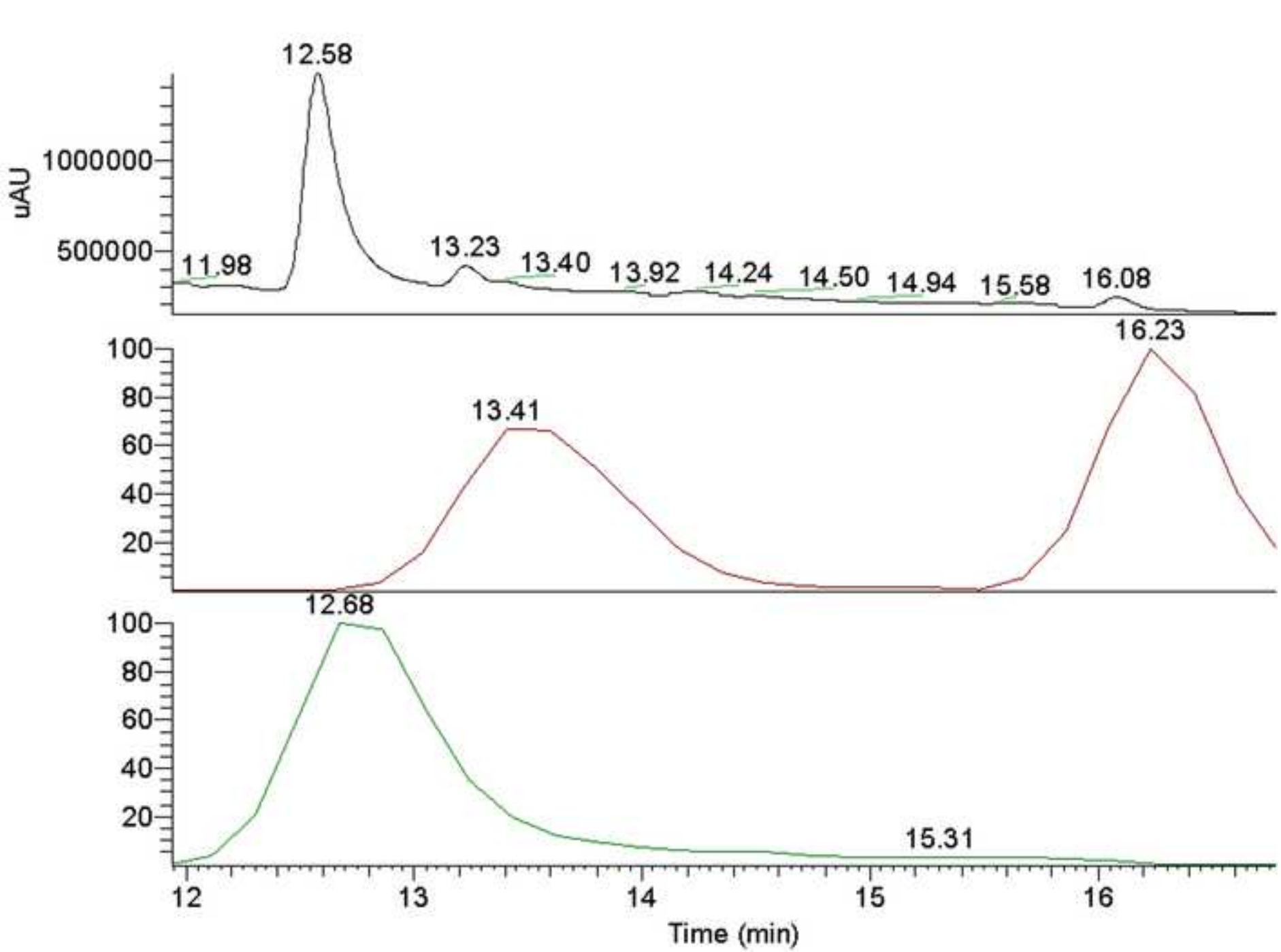

Figure 7a

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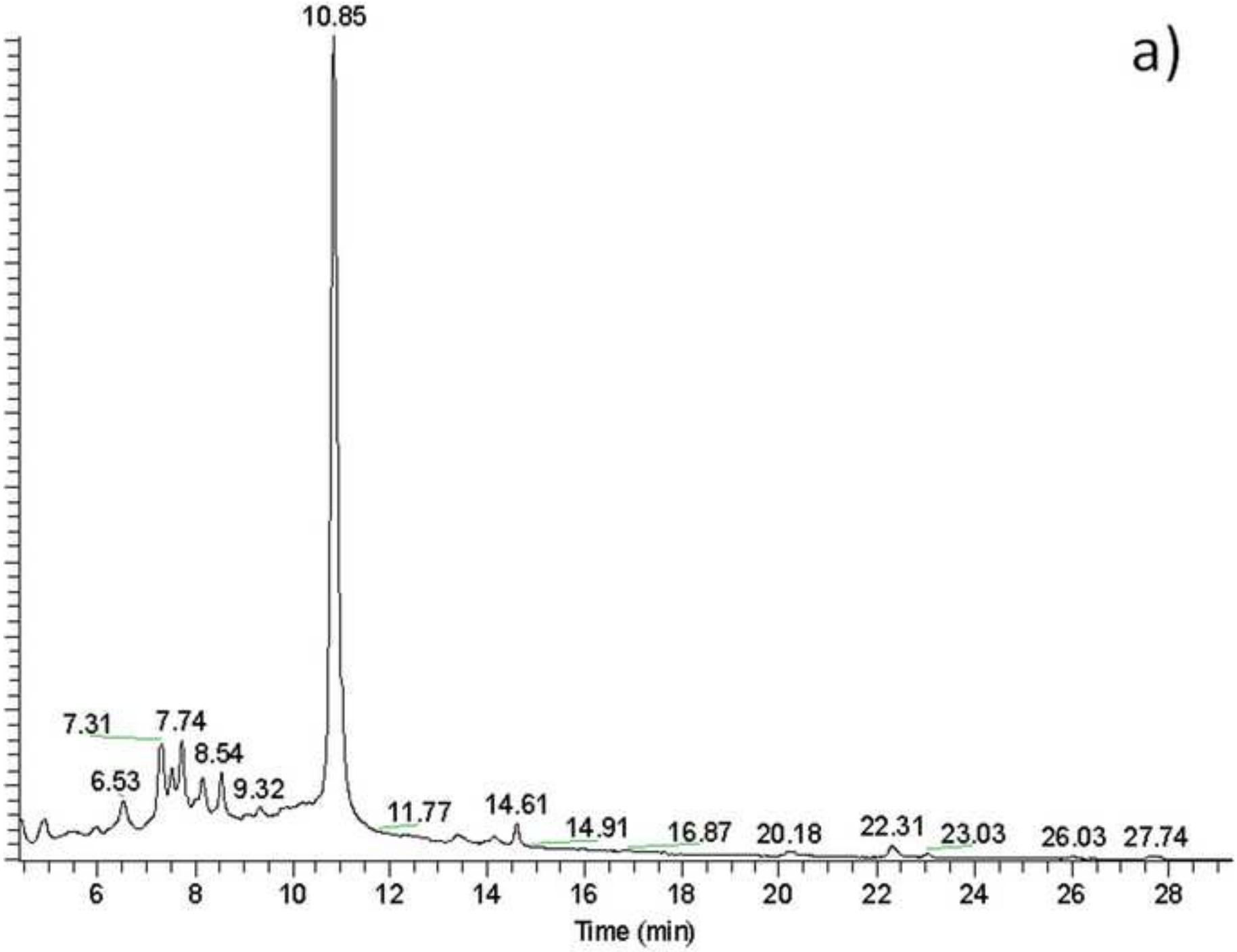

Figure 7b

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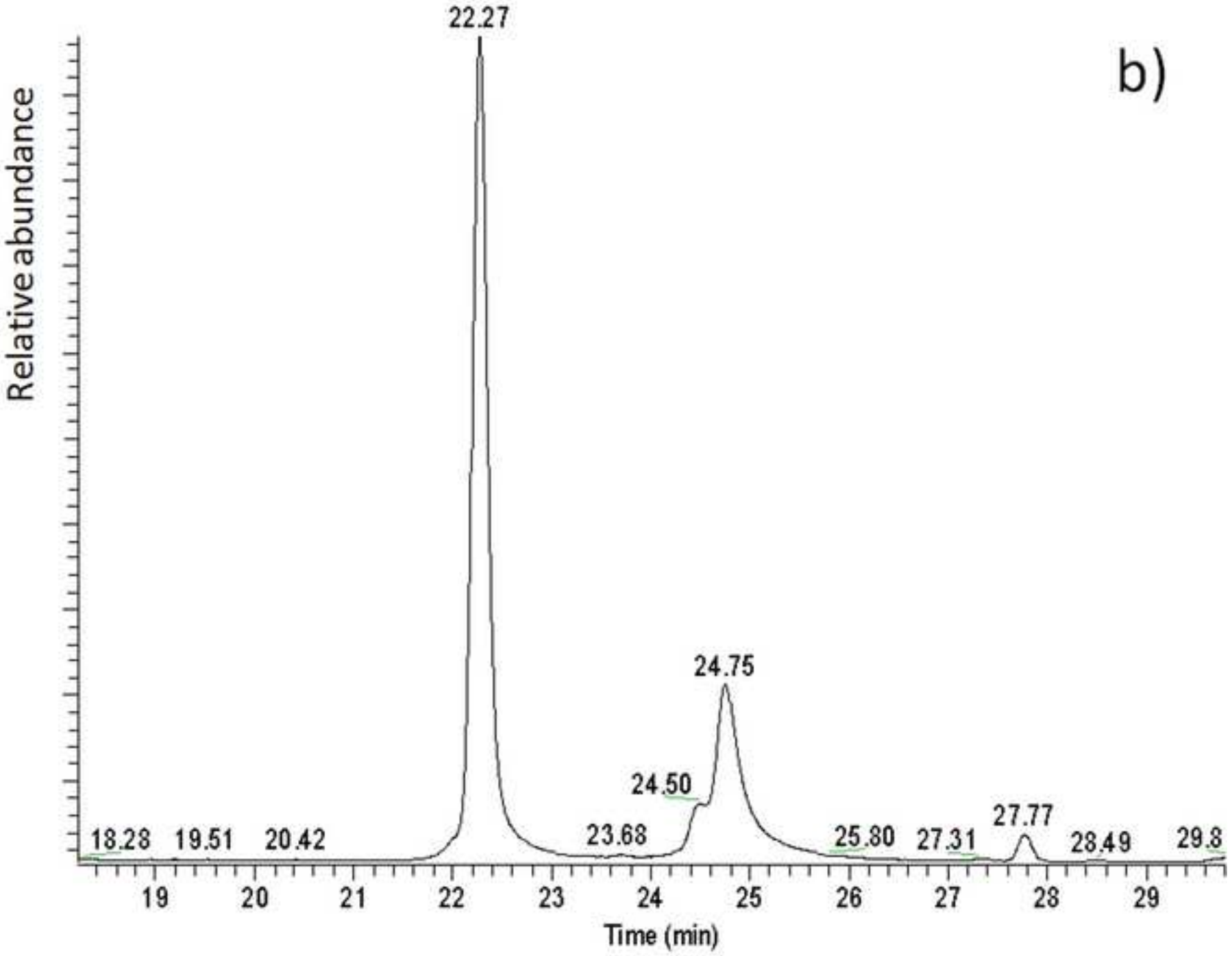

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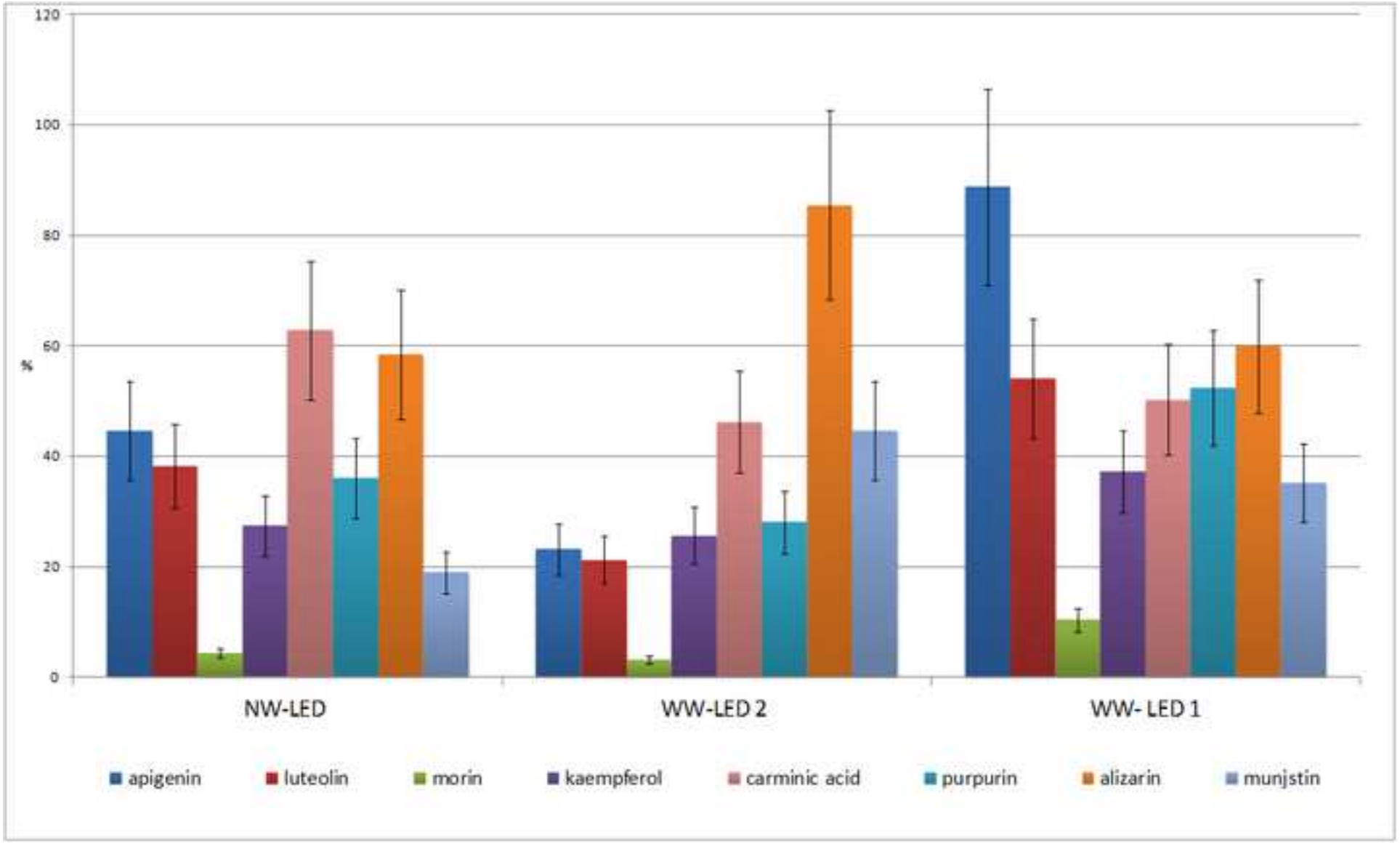

Figure 9

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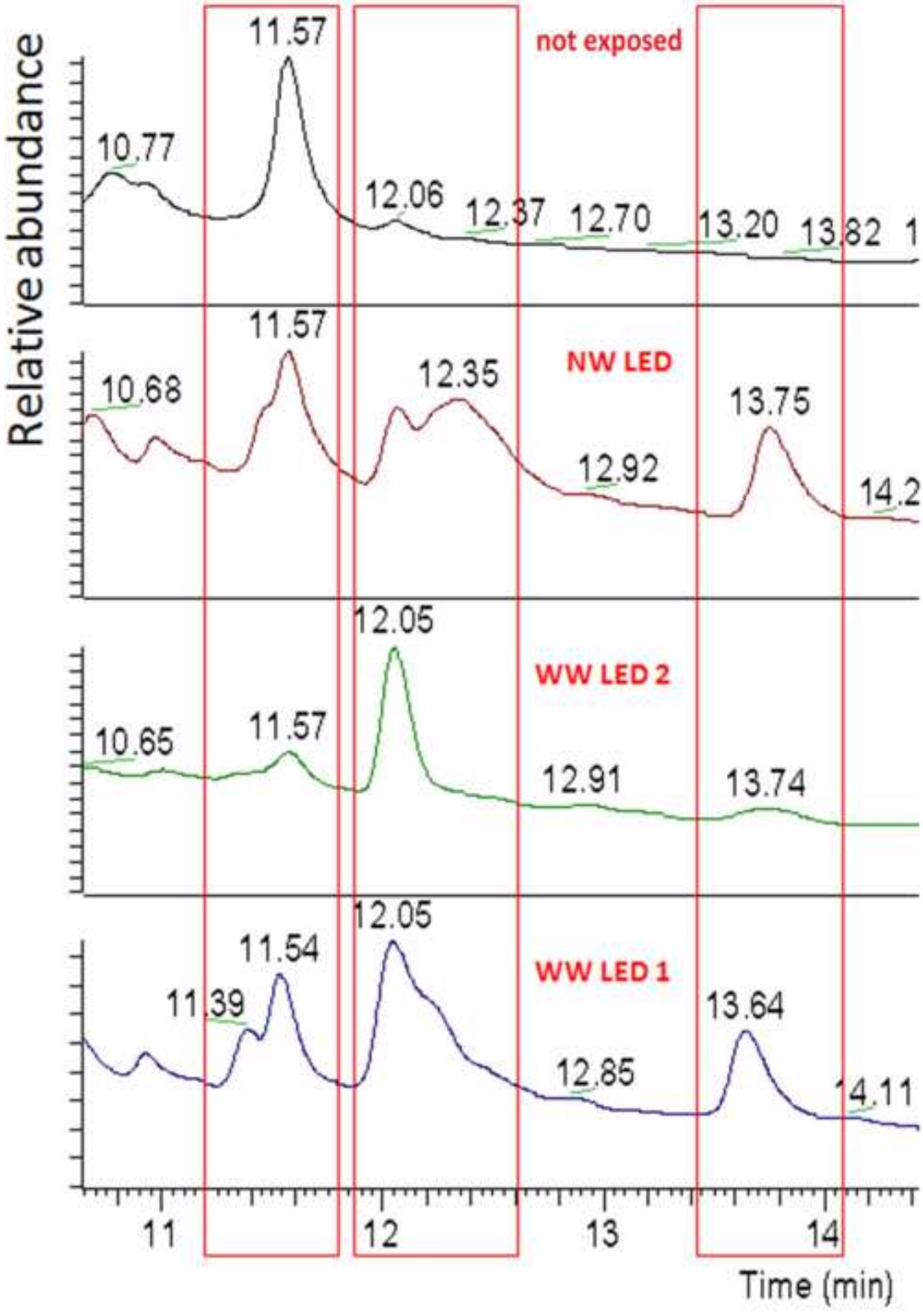

Figure 10

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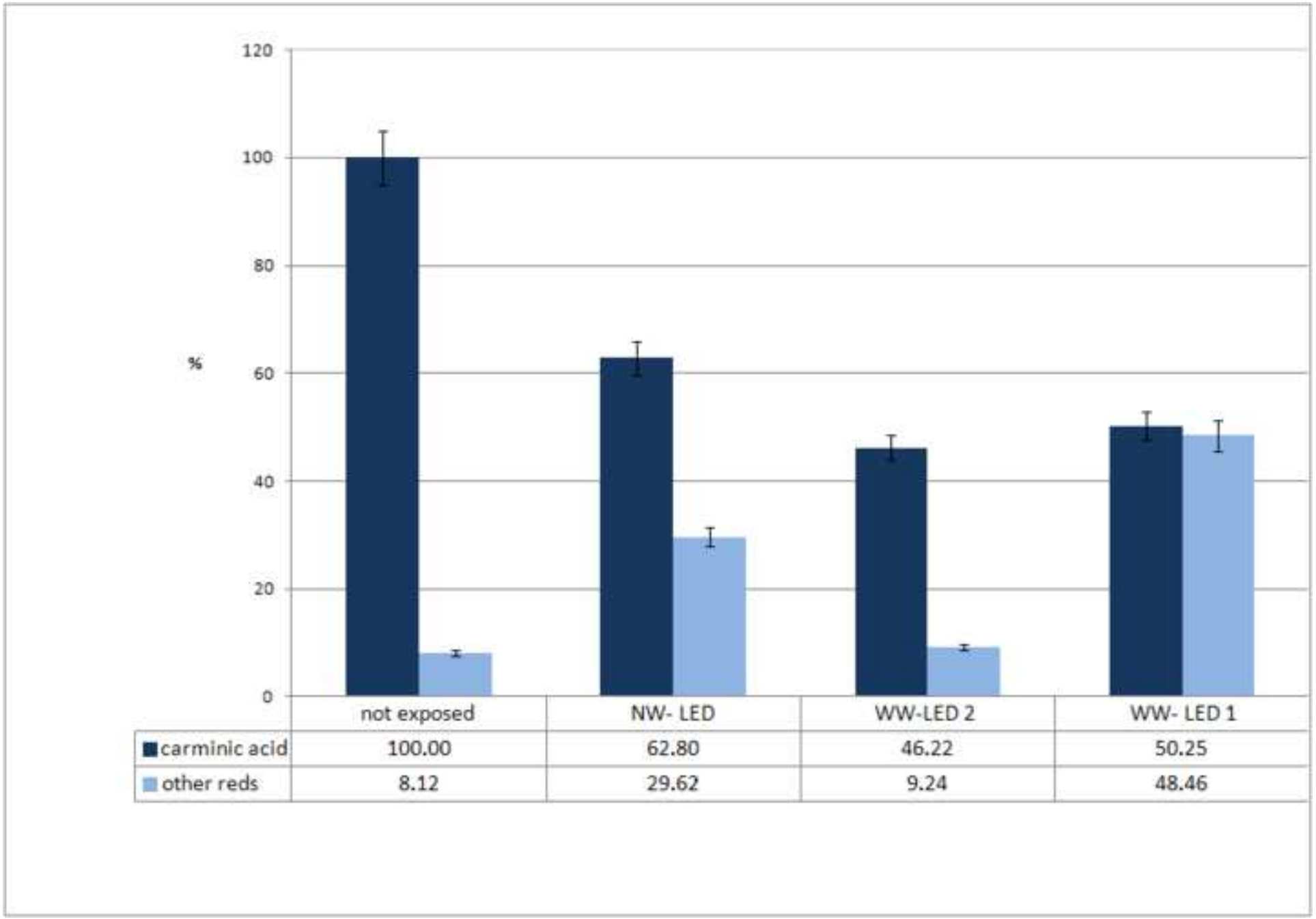

	not exposed	NW- LED	WW-LED 2	WW- LED 1
carminic acid	100.00	62.80	46.22	50.25
other reds	8.12	29.62	9.24	48.46

Table 1: Photo-radiometric characteristics of the investigated LED lamps.

LED type	**CCT [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 features of the molecules detected by HPLC-DAD-MS.

Dye	Compound	Mass weight	Ionization	$[M+H]^+$ (m/z)	MS^2 characteristic fragment ions (m/z)	Absorbance maxima (λ, 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	28.2	39.3	38.4	22.1	44.3	29.9	5.3	4.3