**High Resolution Mass Spectrometry**

The molecular structures of the two references, orcein powder and an extract from a naturally dyed
silk (*Lasallia pustulata*), were obtained by HPLC-HRMS, for more details please see 2.3 and 3.1.1.



**Supplementary Figure 1:** Total ion chromatogram obtained in the HR-ESI positive mode of a commercial orcein power. Extracted ion chromatograms; (**1** and **3**) *β-* and *γ-*aminoorcein, *m/z* 485.1722; (**2**) *α-*aminoorcein, *m/z* 363.1320; (**4** and **5**)*β-* and *γ-*hydroxyorcein, *m/z* 486.1560; (**6**) *α-*hydroxyorcein, *m/z* 364.1179.



**Supplementary Figure 2:** Total ion chromatogram obtained in the HR-ESI positive mode of an extract from a naturally dyed silk (*Lasallia pustulata*). Extracted ion chromatograms: (1 and 3) β- and γ-aminoorcein m/z 485.1772; (2) α-aminoorcein, m/z 363.1320; (4 and 5) β- and γ-hydroxyorcein, m/z 486.1560; (6) α-hydroxyorcein, m/z 364.1160.

**Supplementary Table 1:** Accurate mass measurement data for orcein reference samples acquired by QqTOF using internal calibration.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Peak** | **Rt (min)** | **Measured mass** | **Ion Formula** | **Accurate mass** | **Δ (ppm)** |
| **1** | 8.5 | 485.1722 | C28H25N2O6 | 485.1707 | 3.0 |
| **2** | 9.1 | 363.1320 | C21H19N2O4 | 363.1339 | 5.2 |
| **3** | 9.2 | 485.1720 | C28H25N2O6 | 485.1707 | 2.7 |
| **4** | 9.4 | 486.1559 | C28H24NO7 | 486.1547 | 2.5 |
| **5** | 10.3 | 486.1560 | C28H24NO7 | 486.1547 | 2.6 |
| **6** | 10.5 | 364.1160 | C21H18NO5 | 364.1179 | 5.1 |

**Multi-analytical characterization of purple and pink paints**

Supplementary information for the brazilwood pigments lakes and orcein colour paints.



**Supplementary Figure 3:** Apparent absorption (in grey), excitation and emission spectra (in black) from orcein proteinaceous paint reference (glair, pH=9).



**(a)**

**(b)**

**Supplementary Figure 4:** Molecular characterization of brazilwood lake pigments in the *Ajuda Songbook*: **a)** infrared spectra of the pink colour from fol. 17 (in black) and glair reference (in grey); **b)** apparent absorption spectra of fol. 59 (in black), brazilwood lake reproduction and parchment reference (dashed line) [1].



**(b)**

**(a)**

**Supplementary Figure 5:** Molecular characterization of the purple colour in the *Lineage book*: **a)** infrared spectra of the manuscript from page 12 (in black) and glair reference (in grey); **b)** absorbance spectra of page 12 (in black), orcein dyed silk reference (in grey) and parchment (dashed line).



**(b)**

**(a)**

**Supplementary Figure 6:** Molecular characterization of brazilwood lake pigments in the *winter Breviary, Alc. 54*: infrared **(a)** and excitation and emission **(b)** spectra of the pink colour from fol. 92 (in black) and brazilwood pink from the book of hours *Cofre No. 22* (fol. 76v) (in grey) [2].



**(b)**

**(a)**

**Supplementary Figure 7:** Molecular characterization of brazilwood lake pigments in the *Fernão Vaz Dourado Atlas*: **a)** infrared spectra of the pink colour from map 16 (in black) and protein + polysaccharide reference (in grey), see [1] for spectra of brazilwood lake reference; **b)** excitation and emission spectra from map 16.

**References**

1. Vitorino T, Melo MJ, Carlyle L, Otero V. 2016 New insights into brazilwood manufacture through the use of historically accurate reconstructions. *Stud. Conservat.* **(**DOI: 10.1179/2047058415Y.0000000006)
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