

1 **New insights into the composition of Indian yellow and its use in a Rajasthani**
2 **wall painting**

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4 Diego Tamburini ^{(1)*}, Charlotte Martin de Fonjaudran ⁽²⁾, Giovanni Verri ⁽²⁾, Gianluca Accorsi
5 ⁽³⁾, Angela Acocella ⁽⁴⁾, Francesco Zerbetto ⁽⁴⁾, Amarilli Rava ⁽²⁾, Samuel Whittaker ⁽²⁾, David
6 Saunders ⁽¹⁾, Sharon Cather ⁽²⁾

7
8 ⁽¹⁾ Department of Scientific Research, The British Museum, Great Russell Street, London
9 WC1B 3DG, UK

10 ⁽²⁾ The Courtauld Institute of Art, Somerset House, Strand, WC2R 0RN, London, UK,

11 ⁽³⁾ CNR NANOTEC - Institute of Nanotechnology c/o Campus Ecotekne, University of
12 Salento; Via Monteroni - 73100 Lecce, Italy.

13 ⁽⁴⁾ Department of Chemistry "G.Ciamician", University of Bologna, Via F. Selmi 2, 40126,
14 Bologna, Italy

15
16 * Correspondence should be addressed to Dr. Diego Tamburini

17 Email: Dtamburini@britishmuseum.org ; Tel +44 02073238123

18
19 **Abstract**

20
21 The widespread occurrence of Indian yellow on an early 17th-century wall painting in
22 Rajasthan (India) was initially indicated by photo-induced luminescence imaging of the
23 painted scheme in the Badal Mahal within the Garh Palace (Bundi). The presence of the
24 organic pigment was subsequently confirmed by HPLC-ESI-Q-ToF. The results of a multi-
25 analytical study focusing on two samples from the wall painting and two reference pigments
26 from the British Museum and National Gallery (London, UK) are presented here. The
27 research focused on the possible causes for the different yellow/orange hues observed in
28 the painting samples.

29 Analysis of cross-sections with SEM-EDS revealed similar elemental composition for the
30 Indian yellow paint layers, but different underlying layers, indicating a variation in painting
31 technique. The composition of the Indian yellow samples was investigated by HPLC-ESI-Q-
32 ToF with both positive and negative ionisation. In addition to euxanthic acid and euxanthone,
33 a sulphonate derivative of euxanthone was found to be present in all samples, while relative
34 amounts of the three components varied. Flavonoid molecules—morin, kaempferol,
35 quercetin and luteolin—were also detected in one wall painting sample (characterised by a
36 brighter yellow colour) and not in the sample that was more orange.

37 The optical properties of the samples were characterised by photoluminescence
38 spectroscopy in both solid state and aqueous solution. The contribution of each organic
39 compound to the emission spectrum of Indian yellow in solution was also investigated by
40 time-dependent density functional theory (TDDFT) calculations. Small differences in terms of
41 spectral shift were observed in solid state experiments, but not in solution, suggesting that
42 the spectral differences in the emission spectrum were mostly due to different contributions
43 of solid-state arrangements, most likely driven by π - π stacking and/or hydrogen bonds.
44 However, a slight difference at high energies was observed in the spectra acquired in
45 solution and TDDFT calculations permitted this to be ascribed to the different chemical
46 composition of the samples. Time-resolved measurements highlighted di-exponential lifetime
47 decays, confirming the presence of at least two molecular arrangements. Py(HMDS)-GC-MS
48 was also used for the first time to characterise Indian yellow and the trimethylsilyl derivative
49 of euxanthone was identified in the pyrograms, demonstrating it to be a suitable marker for
50 the identification of the pigment in complex historic samples.

51

52 **Keywords:** Indian yellow; SEM-EDS; photoluminescence; HPLC-MS; Py-GC-MS; wall
53 painting

54

55 1. Introduction

56

57 The widespread occurrence of Indian yellow, a pigment described as a mixture of the
58 magnesium and calcium salts of euxanthic acid [1, 2], on the early 17th-century wall paintings
59 in the Badal Mahal ('Cloud Palace') within the Garh Palace in Bundi (Rajasthan, India) was
60 previously reported by the authors [3]. The painted scheme, dated to 1620-30 CE, is one of
61 the earliest, finest and most complete surviving wall paintings illustrating the artistic
62 patronage of Bundi rulers at that time [4]. The daily life of royal figures is associated with
63 scenes of Hindu mythology centred on Krishna (Figure 1). While Indian yellow is often found
64 on Rajput-Mughal miniature paintings from the late 16th to the 19th century [5, 6], its use in
65 wall paintings was previously unknown. The production method of Indian yellow has been
66 debated since the 19th century but conclusions from the exhaustive review by Ploeger *et al.*
67 [7] points towards the likelihood of a metabolic production pathway involving the collection of
68 urine from cows fed solely on mango leaves, as already reported by Mukharji in the 19th
69 century [8].

70 Most published studies of Indian yellow have centred on its identification in works of art [5, 9-
71 13], but detailed investigations of the physical and chemical characteristics of this organic
72 pigment are rare [3]. However, there is evidence that its description as a mixed Ca and Mg
73 salt of euxanthic acid is over-simplistic. In particular, euxanthone, the aglycone product of

74 euxanthic acid, is reported to occur alongside the acid [1, 10], and a sulphonate derivative of
75 euxanthone has been recently identified [3]. The relative abundances of these components,
76 both in terms of the ratio of euxanthone to euxanthic acid and the concentrations of Ca and
77 Mg, have been reported to vary considerably and it has been speculated that they relate to
78 the purity of the pigment [1]. This variability also appeared to have an influence on the
79 photophysical properties of the material [3], but other factors may play a role, **such as the**
80 **mean crystal size of the particles**. The final outcome of these differences is that Indian yellow
81 pigments sometimes show different yellow hues.

82 In this study we decided to investigate further the possible causes for the variations in the
83 optical characteristics of Indian yellow, as the two samples collected from the wall painting
84 showed slightly different colours.

85 Most analytical techniques are increasingly being adapted to offer portability and non-
86 invasiveness (XRF, FTIR, Raman) [14-16] and, when combined with imaging techniques [12,
87 17-20], can provide very useful information, leading to the identification of materials and
88 aiding in their condition assessment. However, when micro-samples can be taken, the level
89 of detailed chemical information that can be obtained increases significantly, especially for
90 organic materials: the observation and analysis of cross-sections (SEM-EDS) [21, 22]
91 coupled with chromatographic analysis (GC-MS, HPLC-MS, Py-GC-MS) [23] is a powerful
92 combination. When these are integrated with analytical methods that are capable of
93 providing information on both the chemical nature and physical characteristics of materials
94 (steady-state and time-resolved photoluminescence spectroscopy) [24-26], the information
95 obtained is yet more comprehensive.

96 For these reasons, a wide array of techniques was adopted for this study: photo-induced
97 luminescence (PL) imaging and spectroscopy, SEM-EDS, HPLC-MS and Py(HMDS)-GC-MS
98 were applied to investigate the samples from both the wall painting and the reference
99 materials. TDDFT calculations were also used to integrate and confirm some information, as
100 they focused on the contribution of each organic compound to the emission spectrum of
101 Indian yellow and they enabled the emission spectra of the samples to be simulated in
102 aqueous solution. This research highlights the complementary roles played by these
103 analytical and theoretical techniques in bringing new insights into the chemical composition
104 and photophysical properties of Indian yellow.

105

106 **2. Materials and methods**

107

108 **2.1 Samples**

109 The wall painting samples were taken from two different locations on the south wall of the
110 Badal Mahal (Figure 2). Sample S63 was characterised by a lemon yellow paint layer,

111 whereas sample S64 showed a deeper orange-yellow paint layer. Both yellows are
112 extensively used for the geometrical and floral patterns present across the wall.
113 Powdered reference samples of Indian yellow were acquired from the pigment archives of
114 the British Museum (BM) and the National Gallery (NG) in London (see Figure 3).
115 Unfortunately, no information on the provenance or age of the BM and NG reference
116 compounds is available.

117

118 **2.2 Photoluminescence (PL) imaging**

119 The equipment and protocol for PL imaging of the painted scheme is described in Martin de
120 Fonjaudran *et al.* [3]. Further characterisation of the PL properties of the BM and NG
121 reference samples was performed using a customised Nikon D7000 camera, which records
122 emission in the c. 350-1100 nm spectral range. The excitation source consisted of two
123 modified Metz Mecablitz 76 MZ-5 xenon flashtubes at their highest output settings [27]. The
124 modification consisted of the removal of the plastic diffuser and its replacement with a filter
125 holder. The excitation and emission ranges were therefore selected by placing bandpass
126 filters in front of the xenon flashtube and camera lens, respectively. The filters used for UV-
127 induced luminescence in the visible (UV-VISL) and IR ranges (UV-IRL), and visible-induced
128 luminescence in the IR range (VIS-IRL) are summarised in Table 1.

129 The NG and BM samples were placed in a non-luminescent metal holder above a uniform,
130 non-luminescent black surface. A uniform, flat white board covering the entire field of view, a
131 Gretag-Macbeth ColorChecker chart, and a set of lambertian reflectance standards
132 (Spectralon® 99, 50, 25 and 12.5%) were inserted in all images to allow for post-capture
133 processing and correction (light distribution, colour correction, removal of ambient stray
134 reflected light). All images were then processed and corrected using Nip2 software following
135 the protocol described in Dyer *et al.* [28].

136

137 **2.3 Optical microscopy**

138 The wall painting samples were embedded in polyester resin and polished to reveal their
139 stratigraphy. Cross-sections were imaged under normal and UV illumination with a Leica
140 DMRX optical microscope equipped with 10x and 20x PL Fluotar objectives. For UV
141 examination, a mercury light source and a filter cube, with excitation filter BP 340-380 nm,
142 dichroic mirror RKP 400 nm and suppression filter LP 425 nm, were used.

143

144 **2.4 SEM-EDS**

145 The cross-sections were observed uncoated using a Hitachi S-3700N variable pressure (VP)
146 scanning electron microscope. Analysis was performed at low vacuum (40 Pa), 15-20 kV

147 accelerating voltage, with an initial 10 mm working distance. The back-scattered electron
148 (BSE) detector was used in the compositional (COMP) mode.

149 Elemental maps were acquired over four hours on selected areas using 2048 x 2048
150 resolution, 0-20 eV energy range and 50 ms dwell time. AZtecEnergy analysis software
151 (Oxford Instruments) was used to process the data and reconstruct the EDS spectra.

152

153 **2.5 Photophysical properties**

154 Emission and excitation spectra were recorded with an Edinburgh FLS980 spectrometer
155 equipped with a Peltier-cooled Hamamatsu R928 photomultiplier tube (185-850 nm) and a
156 Xenon Lamp (450 W) as light source. The emission lifetimes in the pico- to nanosecond
157 timescale were measured using a single photon counting system (Edinburgh FLS980
158 spectrometer) with a 1 MHz laser diode as excitation source coupled with a Hamamatsu
159 MCP R3809U-50, time resolution 20 ps, as detector. The level of confidence is estimated to
160 be $\pm 8\%$ for τ determinations.

161

162 **2.6 TDDFT calculations**

163 The optical properties of the pigment in aqueous solution were investigated in terms of their
164 molecular components, focusing on the photophysical properties of the first $^1(\pi_{\text{H}} \rightarrow \pi_{\text{L}}^*)$
165 excited state. In an attempt to resolve the emission spectra of Indian yellow samples in
166 solution, quantum mechanical calculations in water were carried out, on the ground and the
167 first $^1(\pi_{\text{H}} \rightarrow \pi_{\text{L}}^*)$ singlet excited states of euxanthone (E), euxanthone sulphonate (SE) and
168 euxanthic acid (EA).

169 All the calculations were performed using DFT [29] and its time-dependent extension,
170 TDDFT [30-32], as implemented in the Gaussian09 suite of packages [33], at the
171 B3LYP/TZVP level of theory [34]. The exchange-correlation functional and basis set used
172 were those selected from the benchmarking analysis run in our previous work to reproduce
173 absorbance properties in a vacuum and in water for the same chromophores [3]. Solvent
174 effects were then introduced by means of the CPCM polarisable conductor model
175 implemented in Gaussian09 [35, 36], with the Pauling cavity set [37] and optimisation of the
176 excited states in water performed using a classical linear-response (LR-CPCM) approach
177 [38, 39]. In order to obtain a more accurate description of the solvent effect in water, the
178 solvent reaction field was also taken into account using, on the optimised LR-CPCM
179 geometries, the state-specific self-consistent procedure model (SS-CPCM), [40, 41], one of
180 the most accurate models yet developed to take into account the variation of the solvent
181 polarisation.

182 The vibrationally-resolved emission spectra were calculated by means of the Franck-Condon
183 (FC) approximation, within the adiabatic Hessian approach, implemented in Gaussian09.
184 This model requires a large computational effort, as the vibrational frequencies of the
185 optimised ground and excited state geometries need to be calculated, analytically and
186 numerically respectively.

187

188 **2.7 HPLC-ESI-Q-ToF**

189 A combined mild extraction procedure was adopted in this study. Approximately 0.1 mg of
190 sample was admixed with 200 μ L DMSO and heated at 80°C for 10 minutes. After
191 centrifugation, the supernatant was transferred into another vial. The residue was admixed
192 with 200 μ L of methanol/acetone/water/0.5M oxalic acid 30:30:40:1 (v/v/v/v) at 80°C for 15
193 minutes. The solution was evaporated under N₂ and reconstituted using 200 μ L of 1:1
194 MeOH/H₂O (v/v). The DMSO extract was combined with the oxalic acid extract and the
195 solution was centrifuged for 10 minutes. The supernatant was transferred to a fresh 250 μ L
196 insert and 5-10 μ L of the solution were injected into the HPLC system.

197 Analyses were carried out using a 1260 Infinity HPLC (Agilent Technologies), coupled to a
198 1100 DAD detector (Hewlett-Packard) and a Quadrupole-Time of Flight tandem mass
199 spectrometer with a 6530 Infinity Q-ToF detector (Agilent Technologies) by a Jet Stream ESI
200 interface (Agilent Technologies).

201 The HPLC conditions were: Zorbax Extend-C18 column (2.1 mm \times 50 mm, 1.8 μ m particle
202 size); 0.4 mL \cdot min⁻¹ flow rate; 5 μ L injection volume for MS experiments and 10 μ L for MSMS
203 experiments; 40°C column temperature. Separation was achieved using a gradient of water
204 with 0.1% formic acid (eluent A) and acetonitrile with 0.1% formic acid (eluent B). The elution
205 gradient was programmed as follows: initial conditions 95% A, followed by a linear gradient
206 to 100% B in 10 min, then held for 2 min. Re-equilibration time for each analysis was 10 min.
207 The ESI operating conditions were: drying gas (N₂, purity >98%): 350°C and 10 L \cdot min⁻¹;
208 capillary voltage 4.0 kV; nebulizer gas 276 kPa; sheath gas (N₂, purity >98%): 375°C and 11
209 L \cdot min⁻¹.

210 High resolution MS and MS/MS spectra were acquired in negative and positive modes in the
211 range 100-1700 m/z. The fragmentor was kept at 150 V, nozzle voltage 1000 V, skimmer 65
212 V, octapole RF 750 V. For the MS/MS experiments, different voltages in the collision cell
213 were tested for Collision Induced Dissociation (CID), in order to maximise the information
214 obtained from fragmentation. The collision gas was nitrogen (purity 99.999%). The data were
215 collected by targeted MS/MS acquisition with an MS scan rate of 1.0 spectra per second and
216 an MS/MS scan rate of 1.0 spectra per second. MassHunter® Workstation Software was
217 used to carry out mass spectrometer control, data acquisition and data analysis.

218

219 **2.8 Py(HMDS)-GC-MS**

220 Analytical pyrolysis was performed using 1,1,1,3,3,3-hexamethyldisilazane (HMDS, chemical
221 purity 99.9%, Sigma Aldrich Inc., USA) as a silylation agent for the *in situ* thermally-assisted
222 derivatisation of pyrolysis products. The instrumentation consisted of a 1500 CDS Pyroprobe
223 1000 Series filament pyrolyser (CDS Analytical, USA) (platinum coil) coupled to a gas
224 chromatograph 6890 (Agilent Technologies, USA) equipped with an HP-5MS fused silica
225 capillary column (30 m × 0.25 mm i.d., 0.25 µm thickness, Agilent Technologies, USA). The
226 GC was coupled with an Agilent 5973 Mass Selective Detector operating in electron impact
227 mode (EI) at 70 eV. Pyrolysis temperatures of 400, 500 and 600°C were tested on reference
228 samples and the interface temperature was 275°C. A pyrolysis temperature of 500°C was
229 adopted for the wall painting samples. Similar amounts (c.100 µg) of sample and HMDS (2
230 µL) were inserted into the centre of the pyrolysis quartz tube with quartz wool and then put
231 into the filament coil. The GC injector was used with a split ratio of 1:10 at 300°C.
232 Chromatographic conditions were as follows: initial temperature 36°C, 10 min isothermal,
233 10°C·min⁻¹ up to 280°C, 2 min isothermal; 20°C·min⁻¹ up to 310°C, 50 min isothermal.
234 Helium (purity 99.995%) was used as carrier gas with a constant flow of 1.0 mL·min⁻¹.

235

236 **3. Results**

237

238 **3.1 Photoluminescence imaging**

239 Photoluminescence imaging of the wall painting has been described previously in Martin de
240 Fonjaudran *et al.* [3] but is briefly presented here to provide context for these additional
241 investigations. Figure 3 (top) shows the visible (right), UV-VISL (middle) and UV-IRL (left)
242 images of the overall area from which sample S64 was taken. Sample S64 is characterised
243 by a yellow-orange colour in the visible image and a deep orange fluorescence in the UV-
244 VISL image (excitation c.280-400 nm and emission c.400-700 nm). Practical limitations of *in*
245 *situ* capture of PL images prevented the photoluminescence at the exact site of sample S63
246 from being recorded; however, *in-situ* observations revealed similar PL properties elsewhere
247 in the painting scheme to the second yellow illustrated in Figure 3, characterised by a paler
248 colour in the visible image and a bright yellow fluorescence in the UV-VISL image. Both
249 yellows show strong emissions in the IR range and appear 'glowing white' against a dark
250 grey background in the UV-IRL image (excitation c. 280-400 nm and emission c.700-1000
251 nm).

252 A more exhaustive PL imaging sequence was performed for BM and NG reference samples.
253 Figure 3 (bottom) illustrates the UV-VISL, UV-IRL and VIS-IRL properties of both reference
254 samples (see Table 1 for details on excitation and emission wavelengths). Post-capture
255 image corrections allowed reliable comparison of colour and photoluminescence intensity

256 between both samples. The colour of both samples is only slightly different in the visible
257 range: the BM sample appears more yellow and the NG sample more orange. Comparing
258 their overall emissions, although the two are similar, the NG sample differs in that it shows a
259 stronger emission in the IR spectral range above 830 nm following UV excitation (see UV-
260 IRL2).

261

262 **3.2 Optical microscopy and SEM-EDS**

263

264 The cross-sections of samples S63 and S64 (Figure 4) revealed that the two areas of the
265 painting were executed using different techniques. In sample S63, the yellow paint layer was
266 applied directly on the lime-based plaster, whereas sample S64 showed a
267 preparation/ground layer between the lime-based plaster and the paint layer, clearly visible
268 in the SEM-EDS maps (Figure 5). FTIR analysis of samples of the plaster (not reported
269 here), showed that it is of dolomitic origin ($\text{CaMg}(\text{CO}_3)_2$). This result was in agreement with
270 EDS analysis, which revealed the presence of Ca and Mg in the plaster layer. Si, Al and
271 traces of K, possibly corresponding to the use of clays, were present as the most abundant
272 elements in the ground/preparation layer of sample S64. Some particles containing lead
273 were also detected by EDS in the white preparation layer. Lead might have been naturally
274 present in the material or added as lead white or as another lead-containing material.

275 When observed at high magnification under UV radiation, S64 appeared to be composed of
276 an orange matrix, in which small bright yellow particles were embedded. This was not
277 observed for sample S63, which showed a more homogeneous distribution of the bright
278 yellow emission. The mean size of the particles was difficult to measure, especially because
279 some larger particles were found to be agglomerates of smaller particles. Nevertheless, as a
280 general observation, finer particles with a more homogeneous distribution were observed in
281 sample S63, whereas larger particles (up to 30 μm) were present in sample S64. This may
282 be related to different methods of manufacture of the pigments, resulting in slightly different
283 colour shades. However, the areas corresponding to particles with different colours did not
284 correspond to any variation in elemental composition, as highlighted in the SEM-EDS
285 images (Figure 4).

286 The paint layer showed the presence of Mg and Ca, in agreement with the composition of
287 Indian yellow [1]. In both samples, Mg appeared to be relatively more abundant and more
288 homogeneously distributed than Ca, which seemed to be more concentrated in specific
289 areas. However, these areas do not correlate with the presence of other specific elements or
290 colours. A similar distribution of Ca and Mg, as well as some variability in the size of the
291 particles, were also observed in the two powdered reference samples (Figures S1 and S2,
292 Supplementary Material).

293

294 **3.2 HPLC-ESI-Q-ToF**

295

296 The principal results obtained for samples NG and S64 have been described briefly
297 previously in Martin de Fonjaudran *et al.* [3] and are integrated here with those obtained for
298 samples BM and S63. HPLC-ESI-Q-ToF analysis revealed that the main organic
299 components in all the samples were euxanthic acid (EA), euxanthone (E) and a sulphonate
300 derivative of euxanthone (SE), as shown in Figure 6, where the Total Ion Chromatograms
301 (TICs) of the BM reference sample and sample S63 are reported.

302 MS and MS/MS experiments, necessary to identify the molecules, were conducted using
303 both negative and positive ionisation modes and the fragment ions present in the MS/MS
304 spectra of E, EA and SE are reported in Table 2. The results obtained with negative
305 ionisation on E and EA are in agreement with those obtained by Otlowska *et al.* [10]. SE was
306 only detected in negative ionisation mode and details of its fragmentation are reported in
307 Martin de Fonjaudran *et al.* [3]. In the positive ionisation mode, E and EA appeared to follow
308 the same fragmentation pathways as in negative ionisation mode.

309 In addition, the chromatographic areas of E, EA and SE were integrated and their sum was
310 calculated for all samples. The percentage ratios between the chromatographic areas of the
311 three components and their sums were then considered (Table 2). Although these values
312 are not quantitative concentrations, the results can still be used to compare relative amounts
313 in the samples. Variations in the composition of Indian yellow, in terms of EA and E content,
314 have been reported in the literature and linked to the quality of the pigment. The best quality
315 is said to contain a high concentration of euxanthic acid, whereas cheaper grades contain
316 elevated amounts of euxanthone [1, 42].

317 Analysis indeed revealed relative amounts of EA, E and SE varying from one sample to
318 another. Sample S63 appeared more similar in composition to the NG reference, whereas
319 sample S64 revealed a higher relative content of SE compared to the other samples.

320 Moreover, some minor components were detected only in sample S63 and were identified as
321 four flavonoid molecules, *i.e.* morin, quercetin, luteolin and kaempferol (Figure 6, inset). In
322 particular, morin and kaempferol were detected with both negative and positive ionisation
323 modes, whereas quercetin and luteolin were only detected in the positive ionisation mode,
324 due to their very low relative abundance and their slightly higher ionisation yield in positive
325 mode. This result underlines the importance of running experiments in both ionisation modes
326 whenever possible.

327 Morin and kaempferol are considered to be the main flavonoid components of cockspur
328 thorn or kayu kuning (*Maclura cochinchinensis*), a plant whose native range extends from
329 northern India and China, through Malaysia and Indonesia into Australia, and which has

330 been used since the 14th century in these parts of the world to produce a yellow dye [43]. It is
331 reported that minor amounts of quercetin and luteolin can be present in the wood [44].
332 Therefore, the Indian yellow in sample S63 appears to be mixed with a yellow lake, probably
333 obtained from kayu kuning extracts and used to create a slightly different yellow shade. To
334 the best of our knowledge, this represents the first analytical evidence of such a mixture,
335 although an account of traditional materials used in Mithila paintings (Bihar, northern India)
336 reports the use of Indian yellow in combination with saffron (*Crocus sativus*) [45].

337

338 **3.3 Photophysical properties**

339

340 The photophysical properties of samples S63 and S64 were investigated in order to identify
341 the key factors influencing their optical features (absorption and emission). The results were
342 compared with those obtained for the reference samples (NG and BM), for which a study in
343 aqueous solution was also possible.

344 Figure 7 reports the solid-state, normalised emission spectra for all the samples ($\lambda_{\text{ex}} = 405$
345 nm). Sample NG showed the highest red-shift emission (λ_{max} at 600 nm), while sample S63
346 showed the highest blue shift ($\lambda_{\text{max}} = 570$ nm). The λ_{max} of samples BM and S64 occur in
347 between those of NG and S63.

348 The corresponding lifetime decays (Figure 7, inset) also showed different values. A mono-
349 exponential decay was obtained for sample S63 ($\tau = 1.5$ ns), whereas di-exponential curves
350 were obtained for samples S64 ($\tau_1 = 0.5$ ns, $\tau_2 = 1.5$ ns), NG ($\tau_1 = 0.5$ ns, $\tau_2 = 1.5$ ns) and BM
351 ($\tau_1 = 0.6$ ns, $\tau_2 = 1.6$ ns). A di-exponential decay generally suggests the presence of different
352 packed domains, which can be driven by the π - π stacking and/or hydrogen bonds commonly
353 occurring in solid-state organic samples [46]. The results show that this phenomenon
354 occurred to some extent in all the samples except sample S63.

355 Different optical features were also found in the excitation spectra (closely related to the
356 absorption spectra) of the NG and BM reference samples (Figure S3, Supplementary
357 Material). The excitation spectra for S63 and S64 could not be measured, due to the small
358 size of the samples. A spectral shift of c.20 nm was observed between the BM and NG
359 samples. This was in agreement with the slight colour difference between the two samples,
360 which was appreciable with a naked eye.

361 To confirm the presence of a di-exponential decay in the NG and BM reference samples,
362 time-resolved measurements were acquired at different emission wavelengths (Figure 8). By
363 measuring the decays at the rise, maximum and tail of the corresponding emission spectra,
364 a progressive inversion of the relative weights between short and long lifetimes was
365 observed (Table 3). This confirmed the presence of two different package domains mostly

366 emitting at the edges of the emission spectrum and being responsible for the optical output
367 of the solid-state samples.

368 However, the differences observed in the optical features of the Indian yellow solid samples
369 may also derive, in principle, from other factors, such as the different chemical composition,
370 in terms of relative proportions of E, EA and SE (section 3.3), and the presence of impurities
371 or additional components in the composition of the pigment (section 3.3).

372 In order to achieve a deeper understanding of these factors, a photophysical study in
373 aqueous solution was performed for samples BM and NG. This allowed us to focus on the
374 effect that a different chemical composition and/or the presence of impurities may have on
375 the optical features, as packed domains do not form in solution. The experiments were not
376 performed on samples S63 and S64 due to the small amount of material available.

377 Figure 9 shows that in both the excitation and emission spectra of sample BM a shoulder
378 appears at longer wavelengths than in sample NG. In principle, this can be due to the slightly
379 different chemical composition of the two samples, as shown by HPLC-ESI-Q-ToF analysis
380 (section 3.3), or to the presence of inorganic impurities in sample BM [47], which are not
381 present in the NG sample. Regardless of its origin, the shift observed between the spectra in
382 the solid-state experiment (Figure 7) did not occur in solution (Figure 9).

383 This suggested that neither the different ratios between E, EA, SE (section 3.3) nor the
384 presence of impurities are the driving forces for the spectral shift observed in the solid-state
385 experiments. Otherwise, a shift should have been observed in both solution and solid-state
386 conditions. Thus, it can reasonably be stated that the formation of different structural
387 arrangements is the main cause for the spectral features/differences observed in the solid-
388 state experiments [48].

389 Although this was proved for both reference samples, we cannot exclude the possibility that
390 the presence of flavonoids might have influenced the shift observed for sample S63.

391 Furthermore, the presence of impurities and/or differences in the relative abundance of
392 various components in the pigment may be indirectly responsible for the different optical
393 properties, as they may be among the driving forces for the formation of the solid-state
394 structure during the preparation of the pigment.

395

396 **3.4 TDDFT results**

397

398 The emission spectra in water of the BM, NG, S63 and S64 samples were calculated and
399 compared to the available experimental data in order to characterise the emission process
400 and rationalise the difference observed at low energies for the BM and NG samples (see
401 Figure 9). Two different models were implemented for the inclusion of the solvent with the
402 polarisable conductor calculation model (CPCM): the linear response (LR-CPCM) and the

403 state specific (SS-CPCM), which consider the solvent in equilibrium with the excited state
404 electron density by means of a self-consistent procedure. The vertical emission wavelengths
405 obtained for the three dominant molecules present in the pigment samples (EA, SE and E)
406 are presented in Table 4, together with their corresponding vertical absorption wavelengths
407 calculated in our previous work [3]. The adiabatic and 0-0 transitions computed with the LR
408 approximation, *i.e.* considering the initial and final states fully equilibrated with the solvent,
409 are also reported. As indicated in section 2.6, the emission processes were considered as
410 $S_1 \rightarrow S_0$ transitions, where S_1 shows a ${}^1(\pi_H \rightarrow \pi_L^*)$ character in water for all the three
411 molecules under investigation, as shown earlier [3]. Vertical emissions for the optimised LR-
412 CPCM and SS-CPCM curves emphasise the importance of solvent equilibration in solution:
413 the SS-CPCM values are closer to those observed experimentally (see Figure 9).
414 The solvent reorganisation energies of the ground state evaluated by means of the SS-
415 CPCM approach were 0.43 eV, 0.50 eV and 0.33 eV for EA, SE and E, showing a higher
416 solvatochromic effect for euxhantone in a polar solvent. In all cases, the LR approach
417 significantly overestimated the vertical emission process in solution, justifying the selection
418 of advanced solvation models, as also indicated in the literature [49].
419 To characterise fully the emission process of the genuine pigment in water, we computed the
420 vibrationally-resolved emission spectra of EA, SE and E by considering the emission as
421 arising from the minima equilibrium solvated structures of the S_1 LR-CPCM curves.
422 Figure 10 presents the vibronic emission spectra of EA, SE and E calculated with the FC
423 approximation as implemented in Gaussian09 providing the calculated state-specific
424 emission energy as a vertical transition. The theoretical emission spectra for EA, SE and E
425 were built up as a sum of 0-0 transitions and simultaneously excited vibrational contributions
426 as derived from Gaussian09 and then convoluted with Gaussian functions, whose FWHMs
427 were chosen to reproduce the final experimental spectra accurately.
428 The maximum peak in the calculated EA emission spectrum, around 456 nm, is mainly due
429 to transitions of single and double simultaneously excited vibrational oscillators, while, in SE
430 and E spectra, the maximum peaks, respectively at 466 and 446, are largely composed of
431 only single vibrational transitions. In all the spectra, the tail at low energies is the result of
432 higher order simultaneously vibrational transitions.
433 The spectrum of E showed the highest intensity and greatest blue-shift, as depicted in Figure
434 11a, this being the most solvated molecule in water. Figure 11b shows a comparison among
435 the total emission spectra for BM (blue line), NG (green line), S63 (black line) and S64 (red
436 line) samples calculated as a sum of the emission spectra of each molecule, scaled for their
437 relative abundance (see Table 2 in section 3.3). Theoretical results were in excellent
438 agreement with the available experimental data for the NG and BM reference compounds,
439 showing a maximum peak in the emission spectra of all the pigment samples around 440 nm

440 and a more pronounced tail at low energies for the BM sample. The difference observed
441 experimentally between NG and BM can be confidently ascribed to the relative composition
442 of the two samples: the higher abundance of E in BM accounts for the higher tail in the
443 spectrum. Based on the good agreement between calculated and experimental spectra of
444 samples BM and NG, we can also assume that the spectra of samples S63 and S64 are
445 accurately reproduced by theory, thus providing additional data that were impossible to
446 obtain experimentally. In addition, no shift of the maximum was observed for these samples
447 and only slight differences in the intensity of the tail were observed, echoing the behaviour of
448 the reference samples and confirming the observations made above.

449

450 **3.5 Py(HMDS)-GC-MS**

451

452 Due to the absence of previous literature on the analysis of Indian yellow by Py-GC-MS, the
453 NG reference sample was analysed to characterise the pigment, identify the main pyrolysis
454 products and choose the optimum pyrolysis temperature. The pyrograms obtained at 400,
455 500 and 600°C showed two main pyrolysis products, namely the mono-trimethylsilyl
456 derivatives of euxanthone and benzoic acid. The bis-trimethylsilyl derivative of euxanthone
457 was also identified with low relative abundance. Figure 12a shows the pyrogram obtained for
458 the NG reference sample at 500°C. The pyrograms obtained at 400 and 600°C are shown in
459 the supplementary material (Figure S4, Supplementary Material).

460 In euxanthic acid, one of the hydroxyl groups of euxanthone is involved in the bond with the
461 glucuronic acid. When thermal energy breaks this bond, the derivatisation of this position is
462 unlikely to occur for kinetic and steric hindrance reasons, thus explaining the high relative
463 abundance of mono-TMS euxanthone. Both mono-TMS and bis-TMS euxanthone can be
464 considered as molecular markers for the presence of Indian yellow and their mass spectra
465 are reported in Figure S5, Supplementary Material.

466 On the other hand, benzoic acid is a non-specific pyrolysis product easily formed during the
467 pyrolysis of most phenolic molecules. In addition to these main pyrolysis products, some
468 products derived from glucuronic acid were identified, such as 2-hydroxypropionic acid, 2-
469 hydroxyacetic acid, hydroxyethylfuran, hydroxymethylcyclopentenone, hydroxypyranone,
470 and other unidentified compounds. These pyrolysis products can also be considered non-
471 specific, as they can easily derive from the pyrolysis of most sugars [50]. Some other non-
472 specific pyrolysis products derived from euxanthone – phenol, benzenacetic acid, 4-
473 hydroxybenzoic acid – were also present. A number of aliphatic monocarboxylic acids with
474 chain lengths from seven to 18 carbon atoms were observed with relative low abundances,
475 but their presence was due to instrumental contamination, as these compounds were also
476 present in the analytical blanks.

477 When testing the optimum pyrolysis temperature, the results showed that the pyrolysis yield
478 of euxanthone decreased as the temperature increased. On the other hand, the relative
479 abundance of benzoic acid increased as the temperature increased, indicating that further
480 decomposition of euxanthone to smaller non-specific molecules is favoured at higher
481 temperatures. At 400°C, the highest relative abundance of euxanthone was obtained
482 compared to benzoic acid. Nevertheless, we decided to use 500°C as the pyrolysis
483 temperature for the wall painting samples, as the initial aim of Py(HMDS)-GC-MS analysis
484 was to obtain some indication of the nature of the binding medium, and 400°C might have
485 been too low to guarantee the pyrolysis of other materials. However, the relative decrease in
486 the pyrolysis yield of euxanthone was acceptable at 500°C (Figure S4, Supplementary
487 Material).

488 The results obtained for samples S63 and S64 generally showed a low relative abundance
489 of pyrolysis products, as can be noticed by the lower signal/noise ratio compared to the
490 reference sample (Figure 12b and 12c). Mono-TMS euxanthone was clearly detected in both
491 samples, proving the technique to be suitable for the identification of Indian yellow in historic
492 samples. Unfortunately, the results did not allow unequivocal identification of the binding
493 medium. Although the detection of aliphatic carboxylic acids, such as palmitic and stearic
494 acids, as the main pyrolysis products could indicate the presence of a lipid material in the
495 samples [51], contamination is also possible, as these carboxylic acids were also present in
496 the analytical blank. The presence of glycerol alongside the carboxylic acids in the pyrogram
497 obtained for sample S63 is a stronger clue to the presence of a triglyceride, but not enough
498 to confirm the presence of oil. Azelaic acid, usually formed by semi-drying or drying oils, was
499 not detected in the samples. The presence of an oil binder may be therefore considered but
500 the amount of sample did not allow for further analysis to confirm this hypothesis. **However,**
501 **the presence of a small amount of binder should not significantly influence the photophysical**
502 **properties of Indian yellow. In fact, emission from a binder would normally fall at lower**
503 **wavelengths [52]. While this would shift the colour of the images towards the blue, it would**
504 **easily be picked up by spectroscopy as a separate emission. Nevertheless, a**
505 **comprehensive study of the photoluminescence of Indian yellow mixed with different binding**
506 **media would represent an interesting topic for future research.**

507

508 **4. Conclusions**

509

510 This work presents an in-depth investigation of two reference and two historical samples
511 (early 17th-century wall painting in the Garh Palace, Bundi, India) containing Indian yellow.
512 The main aim was to identify factors responsible for differences in optical properties, and

513 provide new insights into the photophysical properties of this relatively poorly-studied
514 pigment.

515 The paint layer in the two historic samples appeared different in terms of the distribution,
516 size and colour of the pigment particles, although this did not seem to correspond to any
517 specific elemental difference. All the samples analysed showed slight differences in their
518 chemical composition, mainly related to the relative amounts of EA, E and SE. Only sample
519 S63, the lightest in colour, revealed the presence of additional flavonoids, which are likely to
520 derive from the plant cockspur thorn or kayu kuning (*Maclura cochinchinensis*).

521 The photophysical properties of the samples also showed differences. Solid-state
522 experiments revealed a shift of the maximum in the emission spectra and di-exponential
523 lifetime decays for all the samples except S63. However, experiments in solution did not
524 highlight any shift, suggesting that this is mainly produced by different structural
525 arrangements occurring in the solid state and probably driven by π - π stacking and/or
526 hydrogen bonds. Despite the absence of a shift in the spectra acquired in water, a difference
527 in intensity was observed at low energies. This was ascribed to the different chemical
528 composition of the samples in terms of relative amounts of EA, E and SE by using TDDFT
529 calculations, which also allowed the simulation of the spectra acquired in water for the
530 historic samples. Although conclusive indications of the binding medium were not obtained
531 by Py(HMDS)-GC-MS, Indian yellow was characterised with this technique for the first time
532 and euxanthone was proved to be a valuable biomarker for the identification of the pigment.
533 Finally, this study highlighted the refined and complex use of this pigment by artists
534 producing monumental paintings in Rajasthan in the 17th century. Different yellow hues were
535 achieved through subtle combination of Indian yellow and yellow organic colourants, as well
536 as different underlying preparation layers.

537 This information on the pigment composition, mixtures and layering of materials is extremely
538 important for conservators as they address the preservation and treatment of these culturally
539 significant artefacts; it provides a basis not only to understand colour variations for this
540 particular pigment, but also to monitor possible colour changes through time, whether due to
541 environmental parameters or exposure to conservation materials, and therefore to design
542 and implement informed and safer conservation interventions.

543

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557

558 **References**

559

- 560 [1] N.S. Baer, A. Joel, R.L. Feller, N. Indictor, Indian yellow, in: R.L. Feller (Ed.) Artists'
561 pigments: a handbook of their history and characteristics, Archetype Publications, London,
562 1986, pp. 17-36.
- 563 [2] N. Eastaugh, V. Walsh, T. Chaplin, R. Siddall, Pigment compendium: a dictionary and
564 optical microscopy of historical pigments. , Butterworth-Heinemann, 2008.
- 565 [3] C. Martin de Fonjaudran, A. Acocella, G. Accorsi, D. Tamburini, G. Verri, A. Rava, S.
566 Whittaker, F. Zerbetto, D. Saunders, Optical and theoretical investigation of Indian yellow
567 (euxanthic acid and euxanthone), *Dyes and Pigments*, 144 (2017) 234-241.
- 568 [4] M.C. Beach, *Bundi Fort : a Rajput world.*, The Marg Foundation, Mumbai, India, 2016.
- 569 [5] T.R. Ravindran, A.K. Arora, S. Ramya, R.V. Subba Rao, B. Raj, Raman spectroscopic
570 study of medieval Indian art of 17th century, *Journal of Raman Spectroscopy*, 42 (2011) 803-
571 807.
- 572 [6] I. Reiche, R. Britzke, G. Bukalis, U. Reinholz, H.P. Weise, R.D. Gadebusch, An external
573 PIXE study: Mughal painting pigments, *X-Ray Spectrometry*, 34 (2005) 42-45.
- 574 [7] R. Ploeger, A. Shugar, The story of Indian yellow – excreting a solution, *Journal of*
575 *Cultural Heritage*, 24 (2017) 197-205.
- 576 [8] T.N. Mukharji, Piuri or Indian yellow in, *Journal of the Society of the Arts*, 32 (1883-84)
577 16-17.
- 578 [9] D.L.A. de Faria, H.G.M. Edwards, V. Careaga, N. Walt, M.S. Maier, A definitive analytical
579 spectroscopic study of Indian yellow, an ancient pigment used for dating purposes, *Forensic*
580 *Science International*, 271 (2017) 1-7.
- 581 [10] O. Otlowska, M. Slebioda, M. Wachowiak, M. Sliwka-Kaszynska, Identification and
582 characterization of the Indian Yellow dyestuff and its degradation products in historical oil
583 paint tube by liquid chromatography mass spectrometry, *RSC Advances*, 5 (2015) 48786-
584 48792.
- 585 [11] G.D. Smith, Cow urine, Indian yellow, and art forgeries: An update, *Forensic Science*
586 *International*, 276 (2017) e30-e34.
- 587 [12] E. Isacco, J. Darrah, The Ultraviolet-Infrared method of analysis, a scientific approach to
588 the study of indian miniatures, *Artibus Asiae*, 53 (1993) 470-491.
- 589 [13] J.H. Townsend, The materials of J.M.W. Turner: pigments, *Studies in Conservation*, 38
590 (1993) 231-254.
- 591 [14] K. Janssens, G. Van der Snickt, F. Vanmeert, S. Legrand, G. Nuyts, M. Alfeld, L.
592 Monico, W. Anaf, W. De Nolf, M. Vermeulen, J. Verbeeck, K. De Wael, Non-Invasive and
593 Non-Destructive Examination of Artistic Pigments, Paints, and Paintings by Means of X-Ray
594 Methods, *Topics in Current Chemistry*, 374 (2016) 81.
- 595 [15] M. Manfredi, E. Barberis, A. Rava, E. Robotti, F. Gosetti, E. Marengo, Portable diffuse
596 reflectance infrared Fourier transform (DRIFT) technique for the non-invasive identification of
597 canvas ground: IR spectra reference collection, *Analytical Methods*, 7 (2015) 2313-2322.

- 598 [16] B. Brunetti, C. Miliani, F. Rosi, B. Doherty, L. Monico, A. Romani, A. Sgamellotti, Non-
599 invasive Investigations of Paintings by Portable Instrumentation: The MOLAB Experience,
600 Topics in Current Chemistry, 374 (2016) 10.
- 601 [17] A. Cosentino, Identification of pigments by multispectral imaging; a flowchart method,
602 Heritage Science, 2 (2014) 8.
- 603 [18] G. Verri, The spatially resolved characterisation of Egyptian blue, Han blue and Han
604 purple by photo-induced luminescence digital imaging, Analytical and Bioanalytical
605 Chemistry, 394 (2009) 1011-1021.
- 606 [19] G. Accorsi, G. Verri, A. Acocella, F. Zerbetto, G. Lerario, G. Gigli, D. Saunders, R.
607 Billinge, Imaging, photophysical properties and DFT calculations of manganese blue (barium
608 manganate(vi) sulphate) - a modern pigment, Chemical Communications, 50 (2014) 15297-
609 15300.
- 610 [20] J. Dyer, S. Sotiropoulou, A technical step forward in the integration of visible-induced
611 luminescence imaging methods for the study of ancient polychromy, Heritage Science, 5
612 (2017) 24.
- 613 [21] K. Keune, A. van Loon, J.J. Boon, SEM Backscattered-Electron Images of Paint Cross
614 Sections as Information Source for the Presence of the Lead White Pigment and Lead-
615 Related Degradation and Migration Phenomena in Oil Paintings, Microscopy and
616 Microanalysis, 17 (2011) 696-701.
- 617 [22] C. Higgitt, M. Spring, D. Saunders, Pigment-medium Interactions in Oil Paint Films
618 containing Red Lead or Lead-tin Yellow, National Gallery Technical Bulletin, 24 (2003) 75-
619 95.
- 620 [23] M.P. Colombini, F. Modugno, Organic Materials in Art and Archaeology, Organic Mass
621 Spectrometry in Art and Archaeology, John Wiley & Sons, Ltd, 2009, pp. 1-36.
- 622 [24] G. Accorsi, G. Verri, M. Bolognesi, N. Armaroli, C. Clementi, C. Miliani, A. Romani, The
623 exceptional near-infrared luminescence properties of cuprorivaite (Egyptian blue), Chemical
624 Communications, (2009) 3392-3394.
- 625 [25] D. Comelli, V. Capogrosso, C. Orsenigo, A. Nevin, Dual wavelength excitation for the
626 time-resolved photoluminescence imaging of painted ancient Egyptian objects, Heritage
627 Science, 4 (2016) 21.
- 628 [26] C. Clementi, F. Rosi, A. Romani, R. Vivani, B.G. Brunetti, C. Miliani, Photoluminescence
629 Properties of Zinc Oxide in Paints: A Study of the Effect of Self-Absorption and Passivation,
630 Applied Spectroscopy, 66 (2012) 1233-1241.
- 631 [27] G. Verri, D. Saunders, Xenon flash for reflectance and luminescence (multispectral)
632 imaging in cultural heritage applications, The British Museum Technical Bulletin, 8 (2014).
- 633 [28] J. Dyer, G. Verri, J. Cupitt, Multispectral imaging in reflectance and photo-induced
634 luminescence modes: a user manual, 2013.
- 635 [29] P. Hohenberg, W. Kohn, Inhomogeneous Electron Gas, Physical Review, 136 (1964)
636 B864-B871.
- 637 [30] R.E. Stratmann, G.E. Scuseria, M.J. Frisch, An efficient implementation of time-
638 dependent density-functional theory for the calculation of excitation energies of large
639 molecules, The Journal of Chemical Physics, 109 (1998) 8218-8224.
- 640 [31] R. Bauernschmitt, R. Ahlrichs, Treatment of electronic excitations within the adiabatic
641 approximation of time dependent density functional theory, Chemical Physics Letters, 256
642 (1996) 454-464.
- 643 [32] M.E. Casida, C. Jamorski, K.C. Casida, D.R. Salahub, Molecular excitation energies to
644 high-lying bound states from time-dependent density-functional response theory:
645 Characterization and correction of the time-dependent local density approximation ionization
646 threshold, The Journal of Chemical Physics, 108 (1998) 4439-4449.
- 647 [33] M. Frisch, G. Trucks, H.B. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani,
648 V. Barone, B. Mennucci, G. Petersson, Gaussian 09, revision D. 01, Gaussian, Inc.,
649 Wallingford CT, 2009.
- 650 [34] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, The
651 Journal of Chemical Physics, 98 (1993) 5648-5652.

652 [35] V. Barone, M. Cossi, Quantum Calculation of Molecular Energies and Energy Gradients
653 in Solution by a Conductor Solvent Model, *The Journal of Physical Chemistry A*, 102 (1998)
654 1995-2001.

655 [36] M. Cossi, N. Rega, G. Scalmani, V. Barone, Energies, structures, and electronic
656 properties of molecules in solution with the C-PCM solvation model, *Journal of*
657 *Computational Chemistry*, 24 (2003) 669-681.

658 [37] D.R. Lide, *CRC handbook of chemistry and physics*, CRC Press, Boca Raton, 2005.

659 [38] M. Cossi, V. Barone, Time-dependent density functional theory for molecules in liquid
660 solutions, *The Journal of Chemical Physics*, 115 (2001) 4708-4717.

661 [39] R. Cammi, B. Mennucci, Linear response theory for the polarizable continuum model,
662 *The Journal of Chemical Physics*, 110 (1999) 9877-9886.

663 [40] R. Improta, G. Scalmani, M.J. Frisch, V. Barone, Toward effective and reliable
664 fluorescence energies in solution by a new state specific polarizable continuum model time
665 dependent density functional theory approach, *The Journal of Chemical Physics*, 127 (2007)
666 074504.

667 [41] R. Improta, V. Barone, G. Scalmani, M.J. Frisch, A state-specific polarizable continuum
668 model time dependent density functional theory method for excited state calculations in
669 solution, *The Journal of Chemical Physics*, 125 (2006) 054103.

670 [42] R.B.G. Kew, Indian Yellow, *Bulletin of Miscellaneous Information*, 39 (1890) 45-50.

671 [43] D. Cardon, *Natural Dyes. Sources, Tradition, Technology and Science*, Archetype
672 Publications Ltd., London, 2007.

673 [44] D. Richardson, S. Richardson, *Asian textiles studies*, 2017.

674 [45] V. Jha, Indigenous colours in Mithila (north Bihar): a historical perspective, *Indian*
675 *Journal of History and Science* 37 (2002) 37-55.

676 [46] A.K. Srivastava, A. Singh, L. Mishra, Tuning of Aggregation Enhanced Emission and
677 Solid State Emission from 1,8-Naphthalimide Derivatives: Nanoaggregates, Spectra, and
678 DFT Calculations, *The Journal of Physical Chemistry A*, 120 (2016) 4490-4504.

679 [47] S. Hofener, P.C. Kooijman, J. Groen, F. Ariese, L. Visscher, Fluorescence behavior of
680 (selected) flavonols: a combined experimental and computational study, *Physical Chemistry*
681 *Chemical Physics*, 15 (2013) 12572-12581.

682 [48] S.-L. Zheng, P. Coppens, *Syntheses, Structures, Photoluminescence and Theoretical*
683 *Studies of Xanthone in Crystalline Resorcinarene-Based Inclusion Complexes*, *Chemistry –*
684 *A European Journal*, 11 (2005) 3583-3590.

685 [49] D. Jacquemin, A. Planchat, C. Adamo, B. Mennucci, TD-DFT Assessment of
686 Functionals for Optical 0–0 Transitions in Solvated Dyes, *Journal of Chemical Theory and*
687 *Computation*, 8 (2012) 2359-2372.

688 [50] D. Fabbri, G. Chiavari, Analytical pyrolysis of carbohydrates in the presence of
689 hexamethyldisilazane, *Analytica Chimica Acta*, 449 (2001) 271-280.

690 [51] I. Bonaduce, A. Andreotti, *Py-GC/MS of Organic Paint Binders*, *Organic Mass*
691 *Spectrometry in Art and Archaeology*, John Wiley & Sons, Ltd, 2009, pp. 303-326.

692 [52] G. Verri, A. Keller, F. Piqué, A. Aldrovandi, Ultraviolet-induced luminescence imaging,
693 in: F. Piqué, G. Verri (Eds.) *Organic Materials in Wall Paintings*, The Getty Conservation
694 Institute, Los Angeles, 2015, pp. 76-82.

695

696

697 **Figure 1.** South wall of the Badal Mahal depicting rulers of Bundi with Hindu deities (left).
698 Detail of a hunting scene on the west wall of the Badal Mahal (right). The painting from
699 which the samples were taken is shown in [3] and some details are present in Figure 2.

700

701 **Figure 2.** Detailed views of the south wall of the Badal Mahal showing the location of sample
702 S63 (left) and sample (S64) right. The overall view of the south wall is presented in Martin de
703 Fonjaudran *et al.* [3].

704

705 **Figure 3.** Top: luminescence images of the area from which sample S64 was taken (visible
706 image [left]), UV-VISL [middle] and UV-IRL [right]). In-situ observation of PL properties of
707 sample S63 location showed similar characteristics to the brighter yellow observed in the
708 above images. Bottom: visible, UV-VISL, UV-IRL and VIS-IRL luminescence images of BM
709 (top) and NG (bottom) reference samples – see Table 1 for details of imaging conditions

710

711 **Figure 4.** Images obtained by optical microscopy under UV illumination (top) and scanning
712 electron microscopy in BSE mode (bottom) corresponding to the same region of the cross-
713 sections for samples **a)** S63 and **b)** S64.

714

715 **Figure 5.** EDS maps obtained for an area of the cross-sections of samples **a)** S63 and **b)**
716 S64.

717

718 **Figure 6.** Total Ion Chromatograms obtained by HPLC-ESI-Q-ToF analysis of **a)** BM
719 reference and **b)** S63, showing euxanthone (E), euxanthic acid (EA) and euxanthone
720 sulphonate (SE) as the main components. Inset: Extract Ion Chromatograms showing the
721 flavonoids detected in sample S63. * = background peak.

722

723 **Figure 7.** Main window: Corrected normalised emission spectra of samples NG (olive), S64
724 (red), BM (blue) and S63 (black) excited at 405 nm. Inset: Corresponding lifetime decays,
725 $\lambda_{em} = 600$ nm.

726

727 **Figure 8.** Lifetime decays ($\lambda_{exc} = 405$ nm) of samples BM (upper, blue) and NG (lower, olive)
728 acquired at 530, 560 and 650 nm, corresponding to the rise, maximum and tail of the
729 emission spectra.

730

731 **Figure 9.** Corrected excitation (left, $\lambda_{em} = 600$ nm) and emission (right, $\lambda_{exc} = 405$ nm)
732 spectra of NG (olive) and BM (blue) in aqueous solution. Inset (right window): Corresponding
733 lifetime decays ($\lambda_{exc} = 405$ nm).

734

735 **Figure 10.** Emission spectra computed in water (black solid line) of **a)** EA, **b)** SE and **c)** E
736 molecules and decomposition of the spectra into contributions from the 0-0 transition (0) and
737 the n (n=1,4) simultaneously excited oscillators.

738

739 **Figure 11. a)** Emission spectra calculated in water for E (red line), EA (green line) and SE
740 (blue line); **b)** Emission spectra calculated in water for BM (blue line) and NG (green line)
741 S63 (black line) and S64 (red line) samples.

742

743 **Figure 12.** Total Ion Chromatograms (TICs) obtained by Py(HMD)-GC-MS analysis of **a)** the
744 NG reference sample of Indian yellow, **b)** S63 and **c)** S64. Pyrolysis temperature: 500°C

745

746

747

748 **Table 1.** Band pass filters and related spectral ranges for PL imaging of reference samples

Imaging	Excitation filters	Excitation spectral range	Emission filter	Emission spectral range
UV-VISL	Xnite 330C + Xnite CC1	c. 280-390 nm	Schott KV418 + IDAS UIBAR	c.400-700 nm
UV-IRL1	Xnite 330C + Xnite CC1	c. 280-390 nm	Xnite 715	715-1100 nm
UV-IRL2	Xnite 330C + Xnite CC1	c. 280-390 nm	Xnite 830	830-1100 nm
VIS-IRL1	BP450 + Xnite CC1	c. 420-480 nm	Xnite 715	715-1100 nm
VIS-IRL2	BP525 + Xnite CC1	c. 490-560 nm	Xnite 715	715-1100 nm
VIS-IRL3	BP625 + Xnite CC1	c. 590-680 nm	Xnite 715	715-1100 nm

749

750

751

752 **Table 2.** Mass spectrometry data obtained with positive and negative ionisation modes and
 753 percentage areas calculated for Indian yellow components euxanthone (E), euxanthic acid
 754 (EA) and euxanthone sulphonate (SE). Standard deviations refer to triplicates.

		[M] ⁻	[M] ⁺	Positive fragment ions	Negative fragment ions	A% _{BM} ref	A% _{NG ref}	A% _{S63}	A% _{S64}
E	C ₁₃ H ₈ O ₄	227.0350	229.0495	211, 201, 183, 173, 155, 127,	199, 181, 171, 155, 143, 117, 91, 79	21.5 ± 0.3	7.9 ± 0.2	3.6 ± 0.1	5.6 ± 0.1
EA	C ₁₉ H ₁₆ O ₁₀	403.0671	405.0816	229, 201, 141, 113, 85	227, 199, 171, 113, 85	71.3 ± 0.2	77.4 ± 0.8	81.5 ± 0.2	65.4 ± 0.4
SE	C ₁₃ H ₈ O ₇ S	306.9918	ND*	ND*	227, 199, 171, 143, 117	7.3 ± 0.2	14.7 ± 0.6	14.9 ± 0.1	28.9 ± 0.4

755 * ND = not detected

756

757

758 **Table 3.** Lifetime decays and relative weights (%) of BM and NG acquired at different
759 wavelengths.

	$\lambda_{em} = 530 \text{ nm}$	$\lambda_{em} = 560 \text{ nm}$	$\lambda_{em} = 650 \text{ nm}$
BM	$\tau_1 = 0.6 \text{ ns (18\%)}$ $\tau_2 = 1.6 \text{ ns (82\%)}$	$\tau_1 = 0.6 \text{ ns (49\%)}$ $\tau_2 = 1.6 \text{ ns (51\%)}$	$\tau_1 = 0.6 \text{ ns (74\%)}$ $\tau_2 = 1.6 \text{ ns (27\%)}$
NG	$\tau_1 = 0.5 \text{ ns (53\%)}$ $\tau_2 = 1.5 \text{ ns (47\%)}$	$\tau_1 = 0.5 \text{ ns (87\%)}$ $\tau_2 = 1.5 \text{ ns (13\%)}$	$\tau_1 = 0.5 \text{ ns (94\%)}$ $\tau_2 = 1.5 \text{ ns (6\%)}$

760

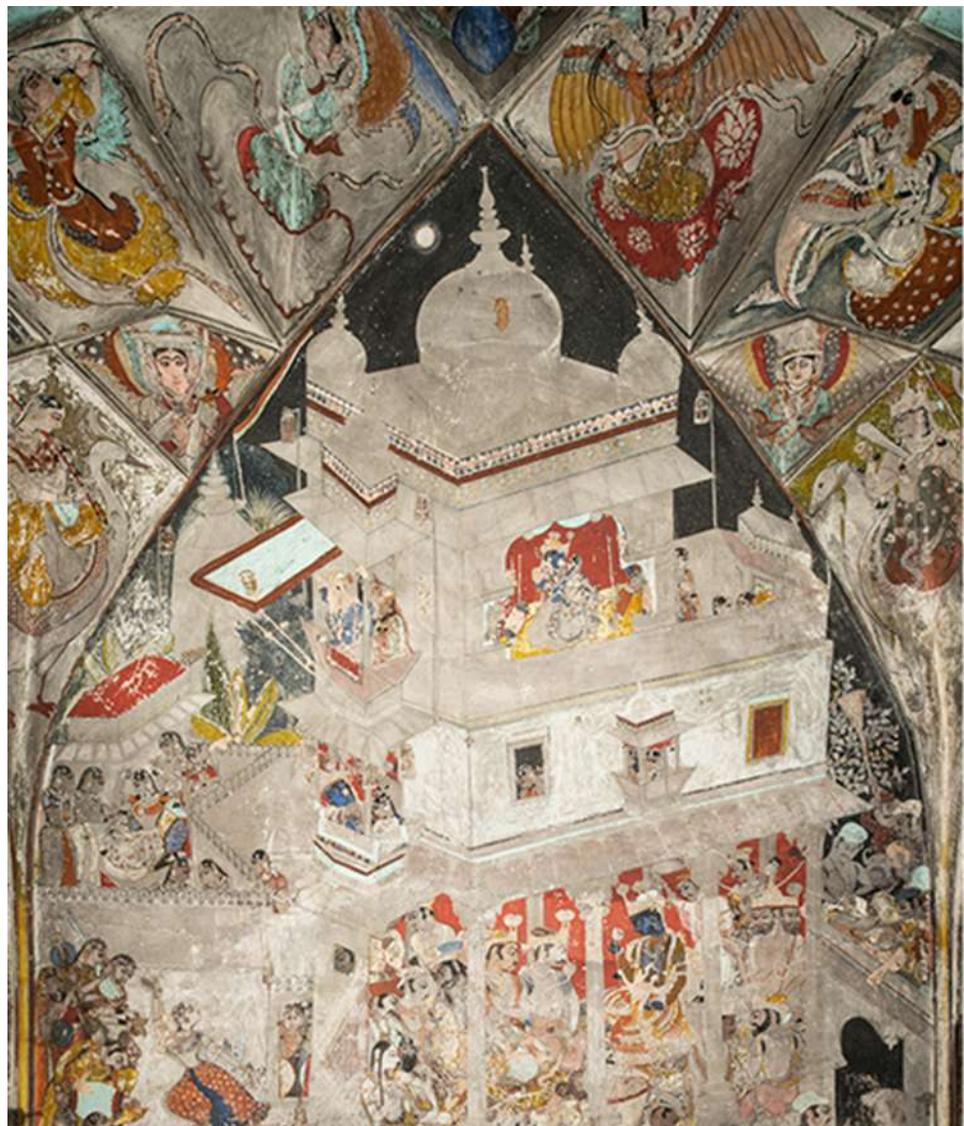
761

762

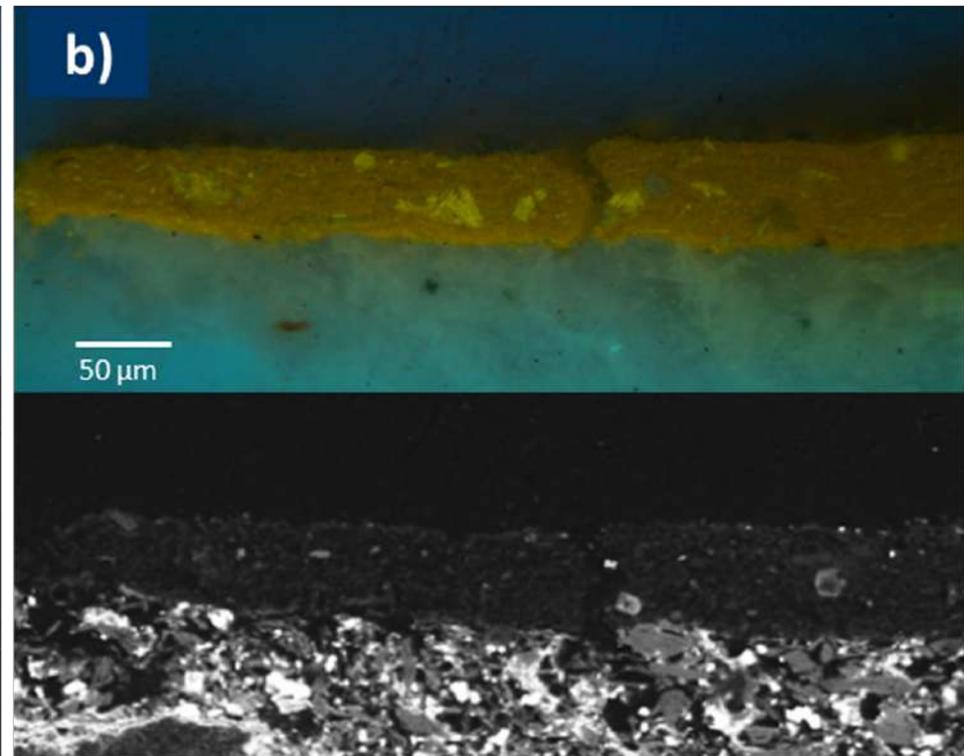
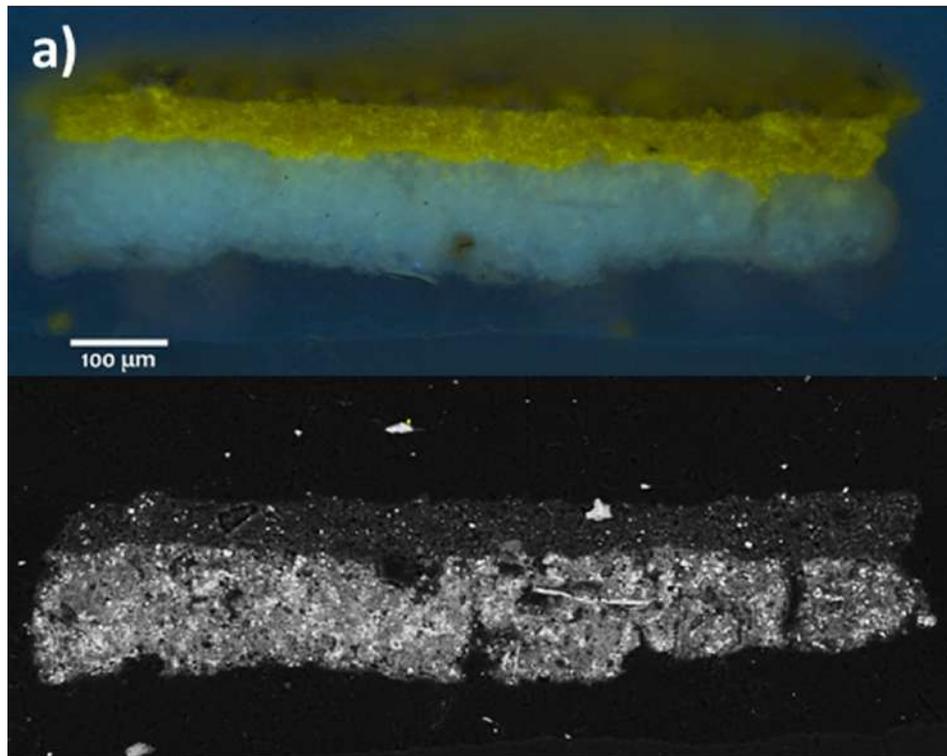
763 **Table 4.** Calculated vertical absorption energies (ΔE_{Abs}), vertical emission energies in LR
 764 approximation ($\Delta E_{\text{Em}}^{\text{(LR)}}$), vertical emission energies in SS approximation ($\Delta E_{\text{Em}}^{\text{(SS)}}$), adiabatic
 765 ($\Delta E_{\text{Em}}^{\text{(ad)-LR}}$) and 0-0 ($\Delta E_{\text{Em}}^{\text{0-0-LR}}$) transition energies in water at the B3LYP/TZVP level of
 766 theory for EA, SE and E compounds. All energies are reported in eV (nm).

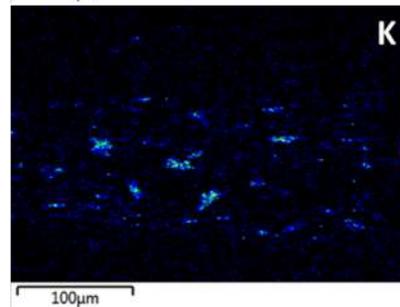
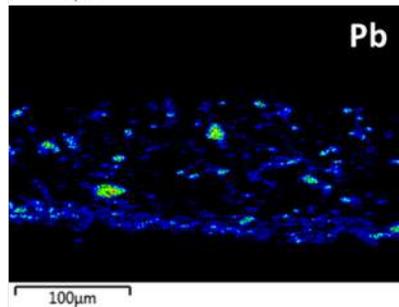
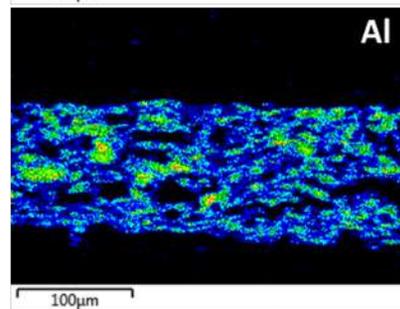
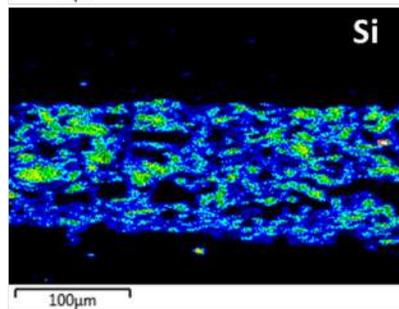
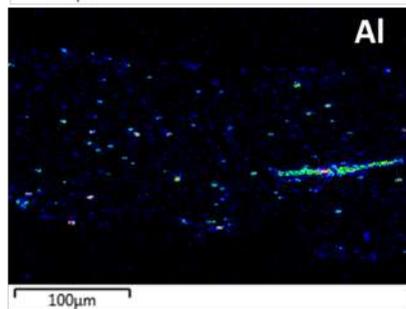
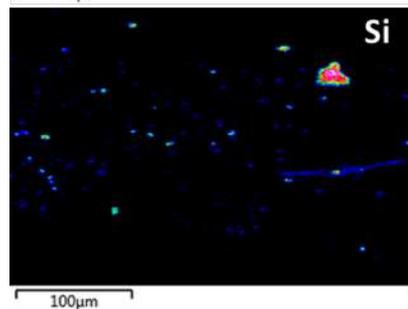
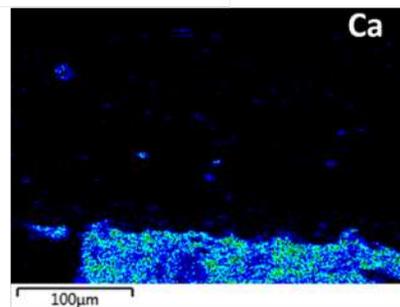
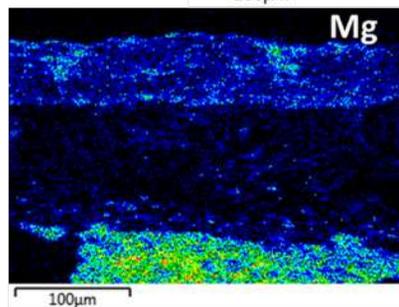
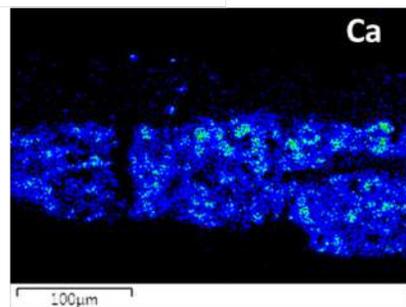
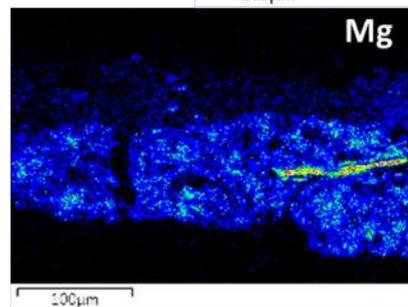
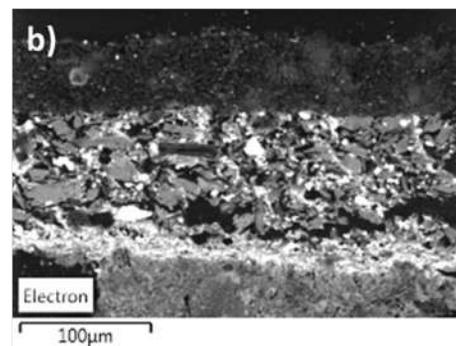
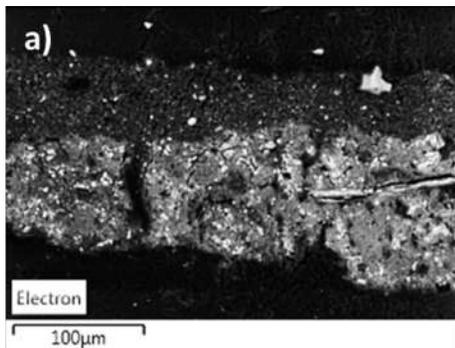
	$\Delta E_{\text{Abs}}^{\text{a}}$	$\Delta E_{\text{Em}}^{\text{(LR)}}$	$\Delta E_{\text{Em}}^{\text{(SS)}}$	$\Delta E_{\text{Em}}^{\text{(ad)-LR}}$	$\Delta E_{\text{Em}}^{\text{0-0-LR}}$
EA	3.41 (363)	2.98 (416)	2.71 (456)	3.14 (394)	3.03 (408)
SE	3.45 (359)	2.94 (421)	2.66 (466)	3.15 (393)	3.06 (404)
E	3.44 (360)	2.94 (421)	2.77 (446)	3.11 (398)	3.00 (412)

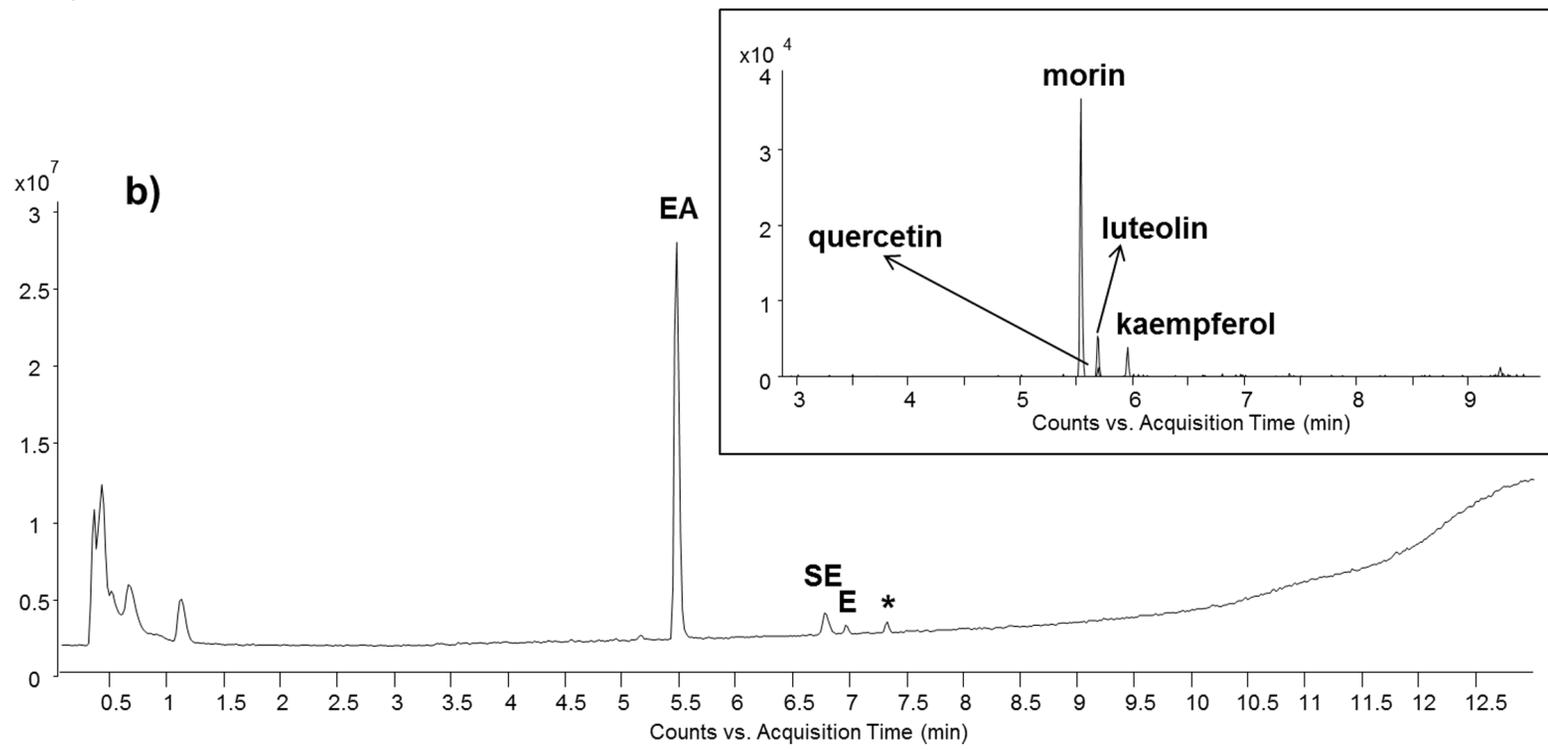
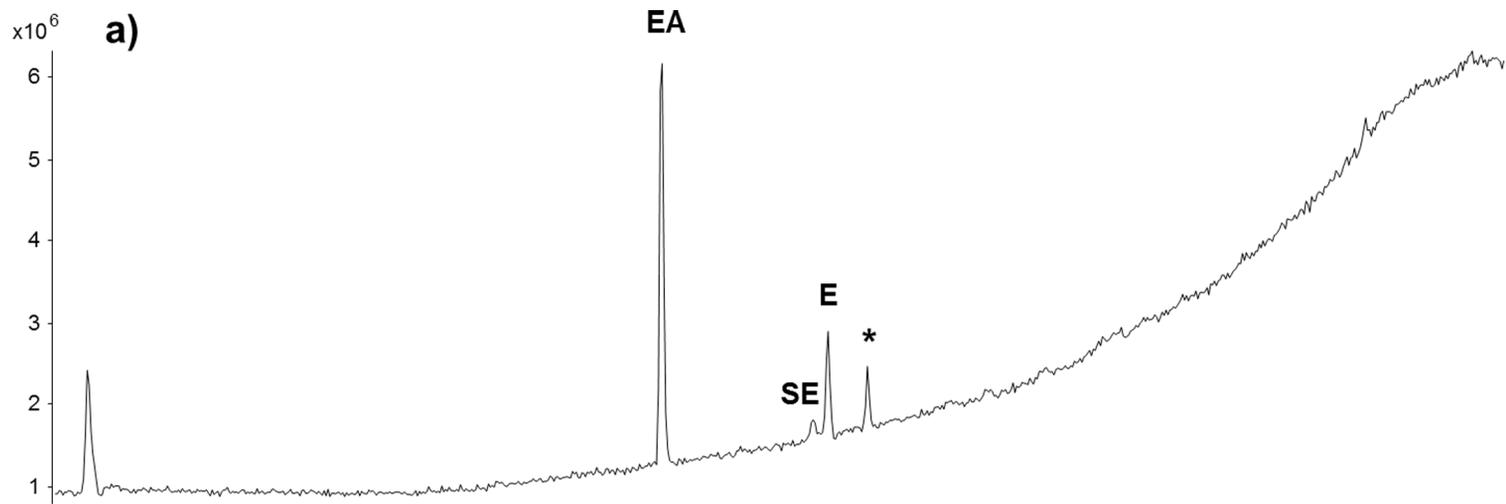
767 ^a as described in [3]

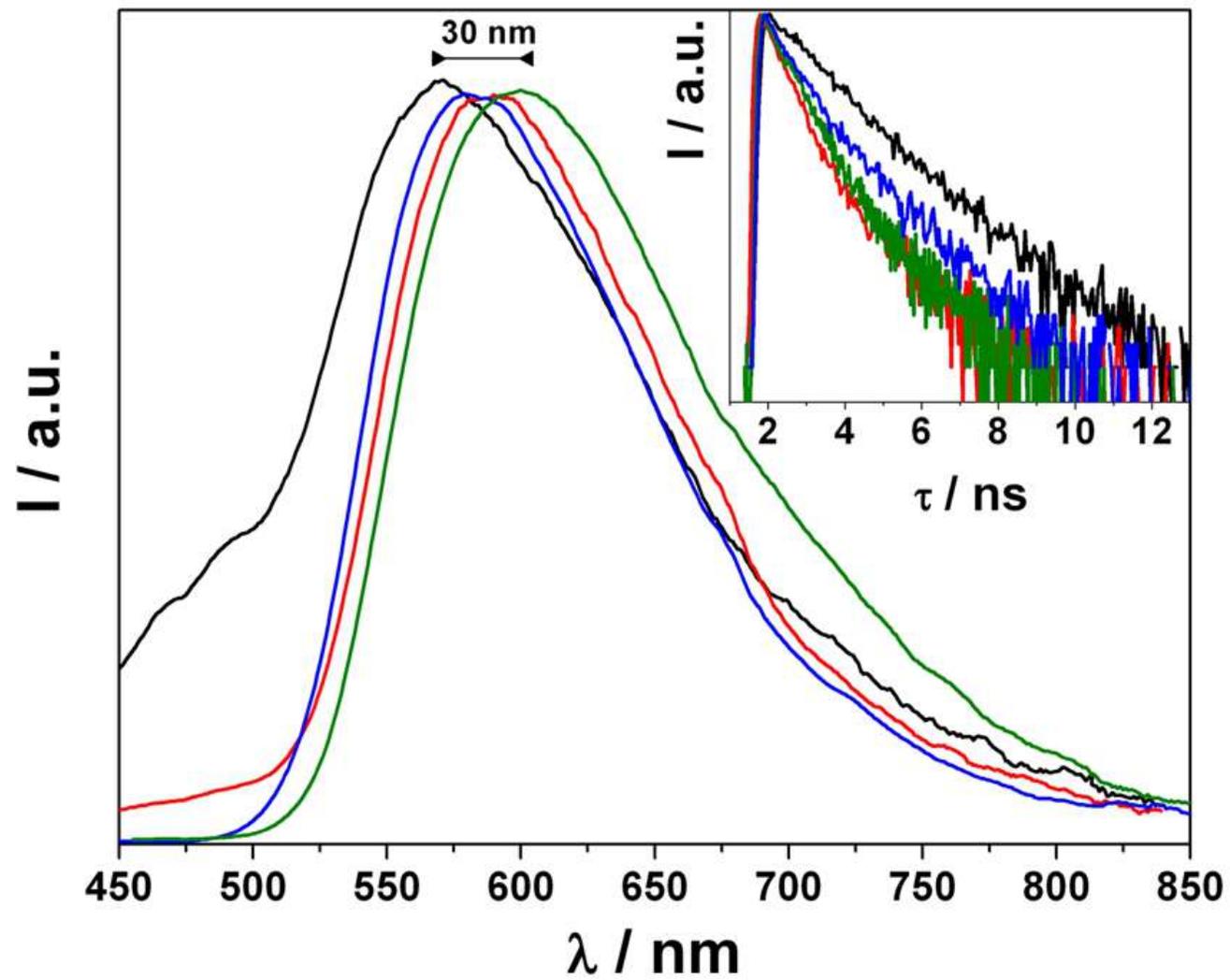


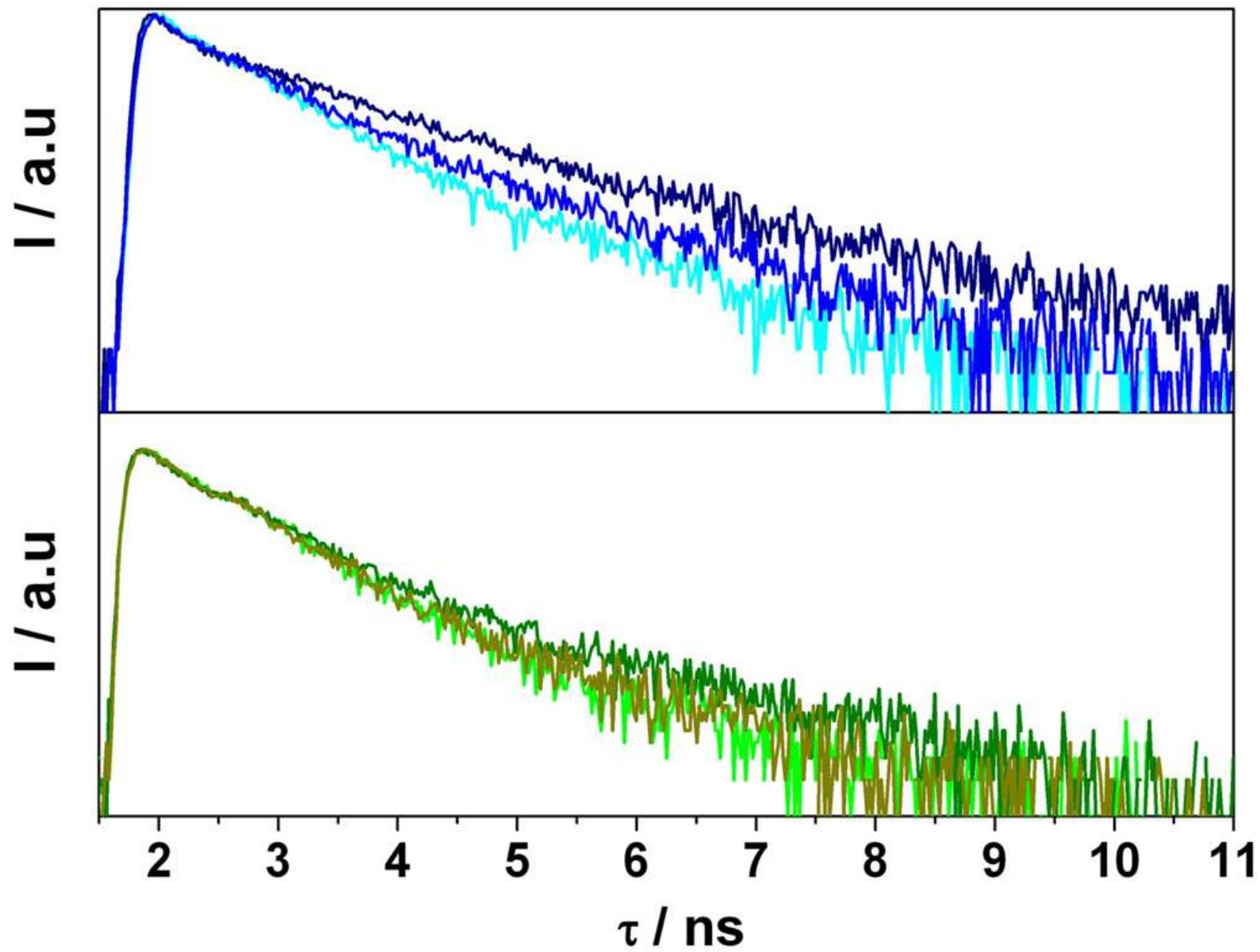


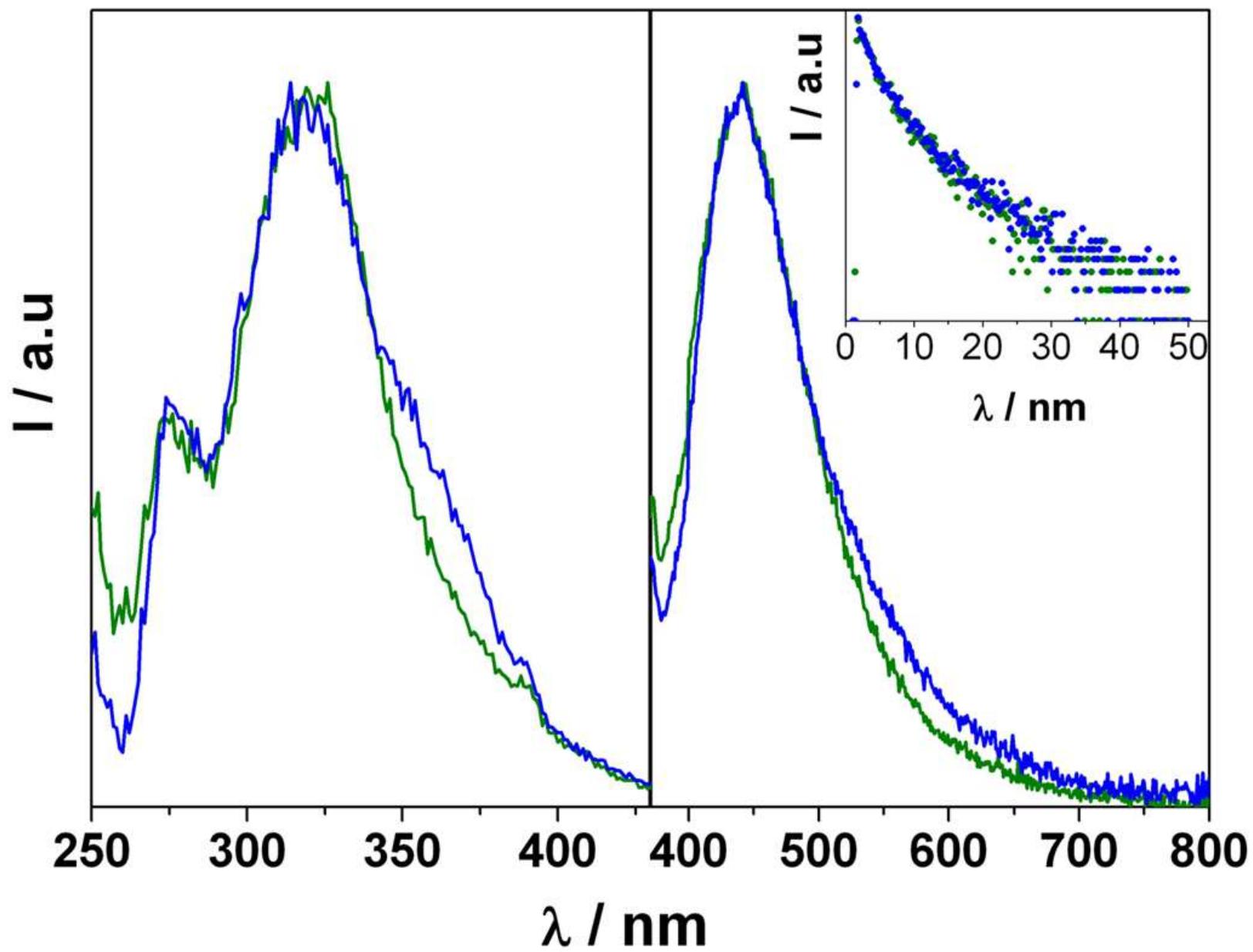


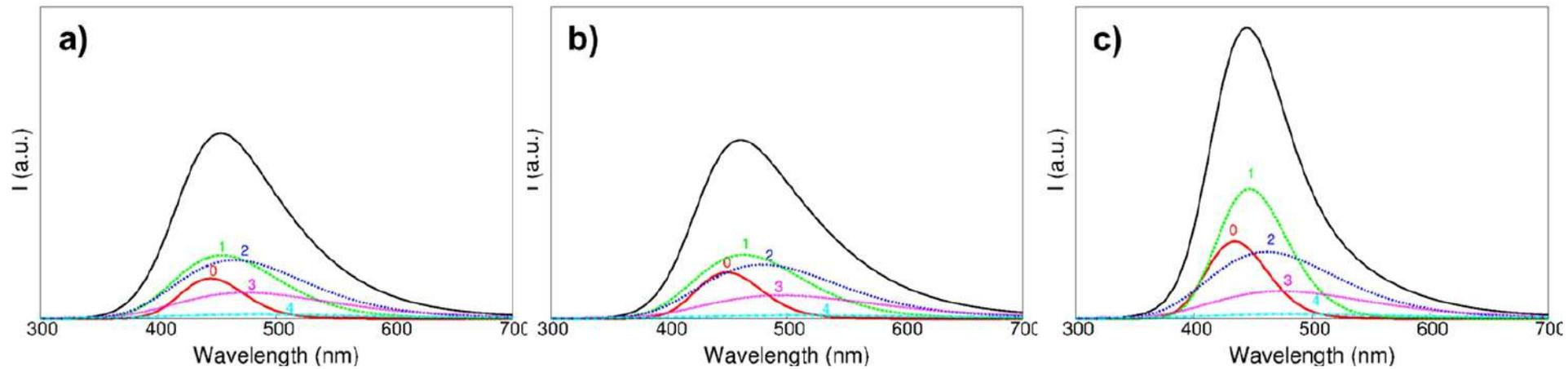


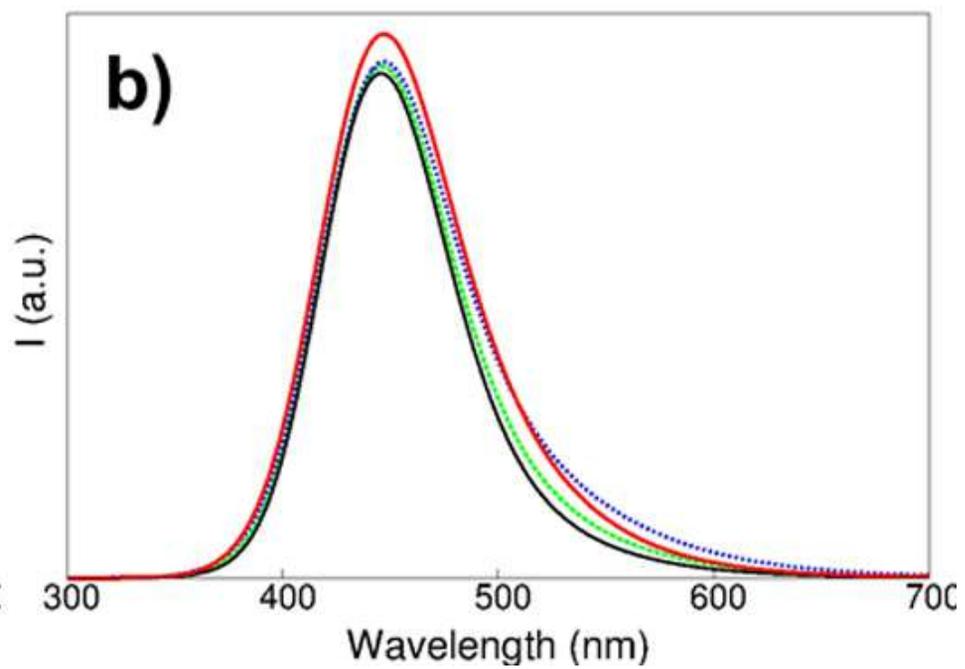
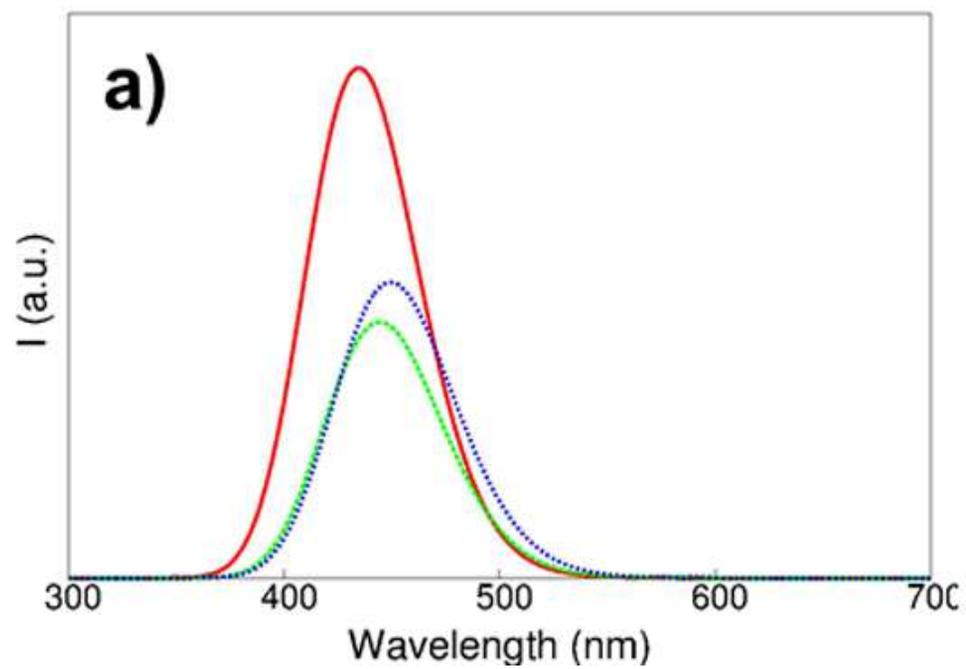


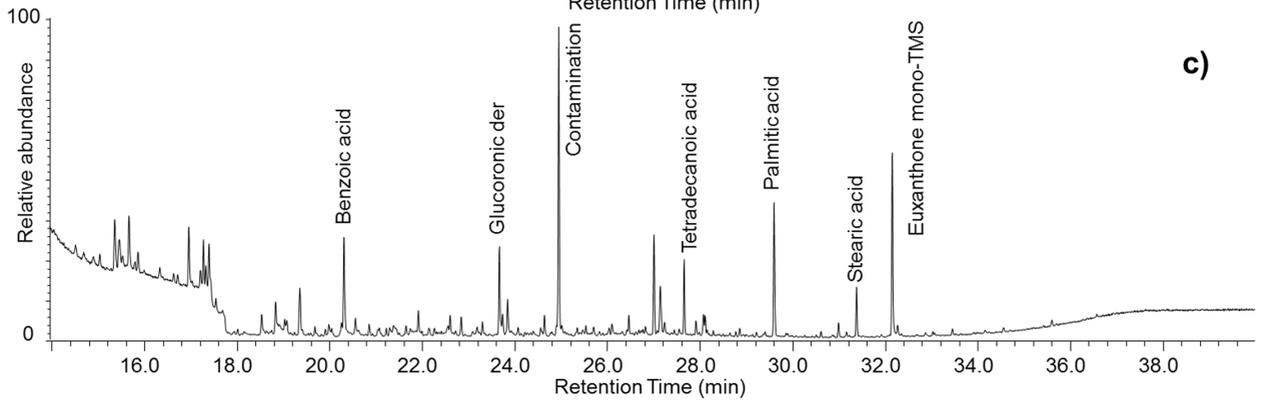
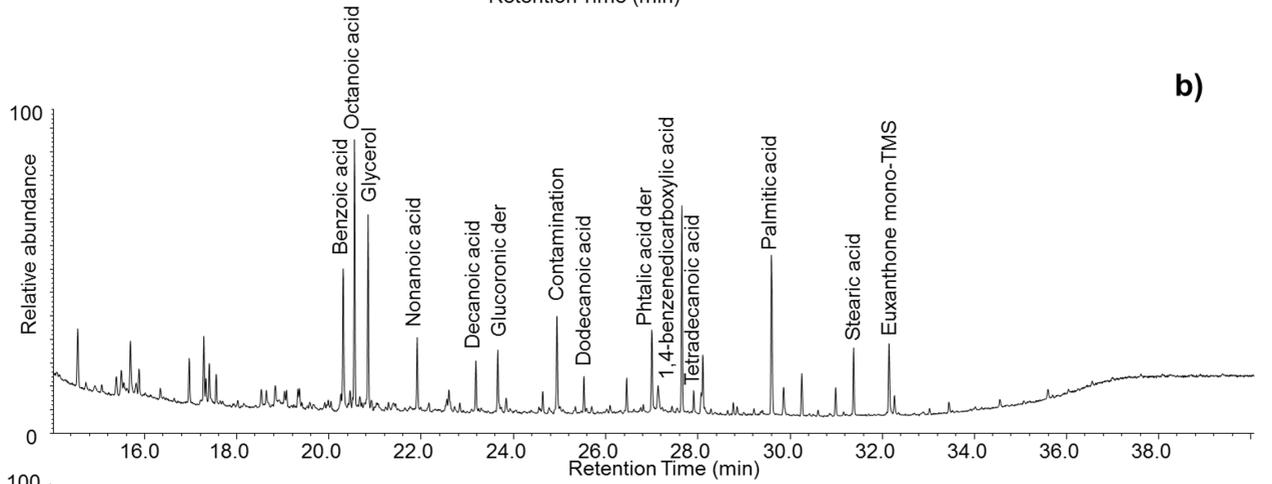
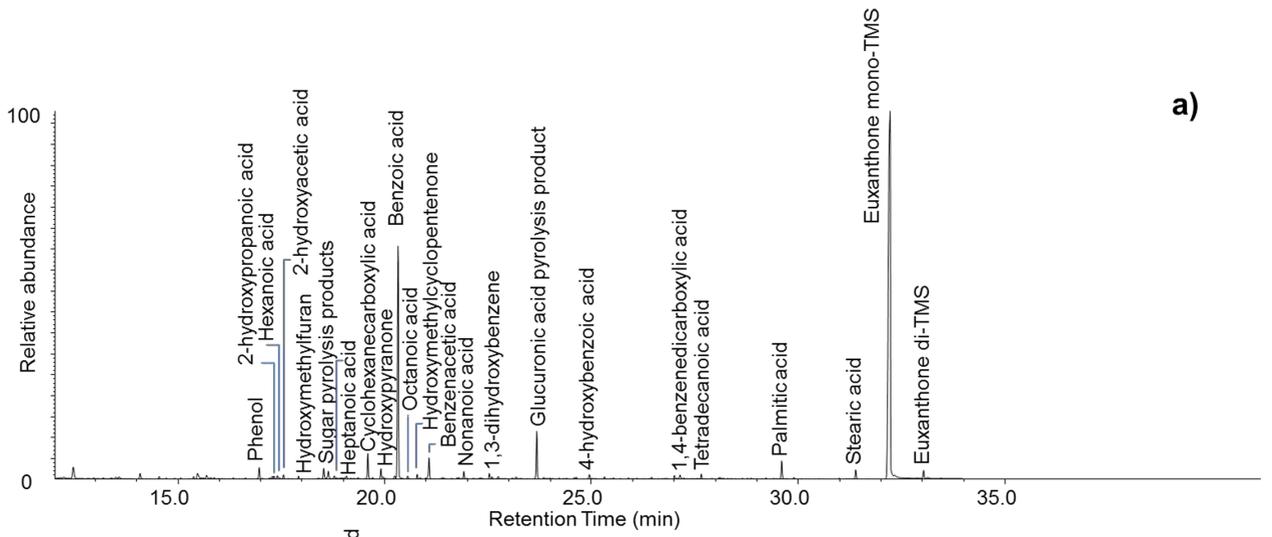












New insights into the composition of Indian yellow and its use in a Rajasthani wall painting

Diego Tamburini ⁽¹⁾, Charlotte Martin de Fonjaudran ⁽²⁾, Giovanni Verri ⁽²⁾, Gianluca Accorsi ⁽³⁾, Angela Acocella ⁽⁴⁾, Francesco Zerbetto ⁽⁴⁾, Amarilli Rava ⁽²⁾, Samuel Whittaker ⁽²⁾, David Saunders ⁽¹⁾, Sharon Cather ⁽²⁾

⁽¹⁾ Department of Scientific Research, The British Museum, Great Russell Street, London WC1B 3DG, UK

⁽²⁾ The Courtauld Institute of Art, Somerset House, Strand, WC2R 0RN, London, UK,

⁽³⁾ CNR NANOTEC - Institute of Nanotechnology c/o Campus Ecotekne, University of Salento; Via Monteroni - 73100 Lecce, Italy.

⁽⁴⁾ Department of Chemistry "G.Ciamician", University of Bologna, Via F. Selmi 2, 40126, Bologna, Italy

Supplementary material

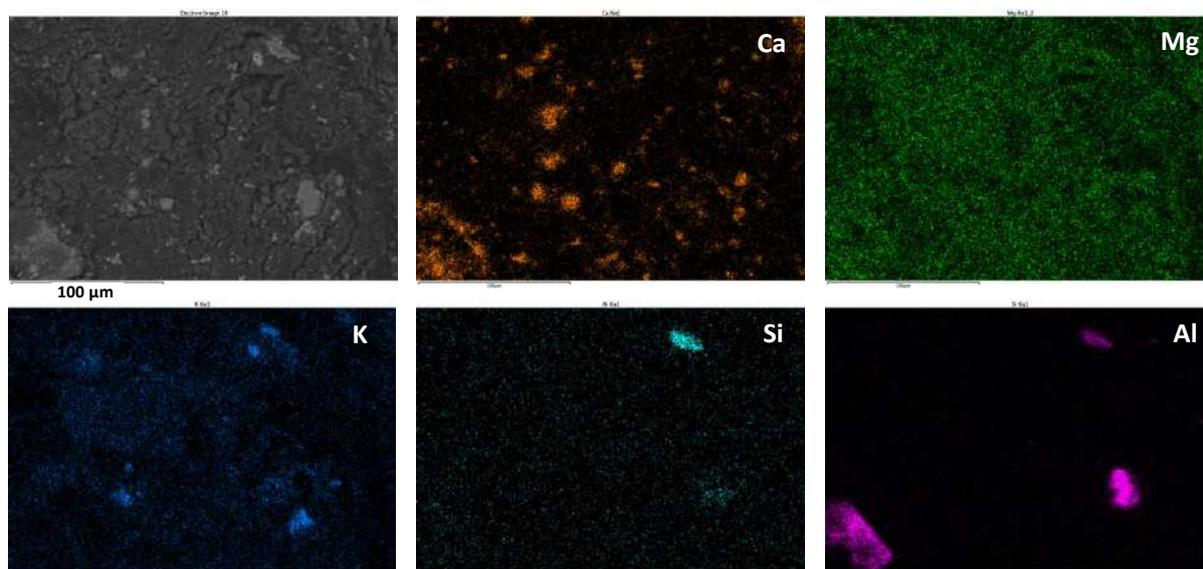


Figure S1. EDS maps obtained for the surface of the NG reference sample.

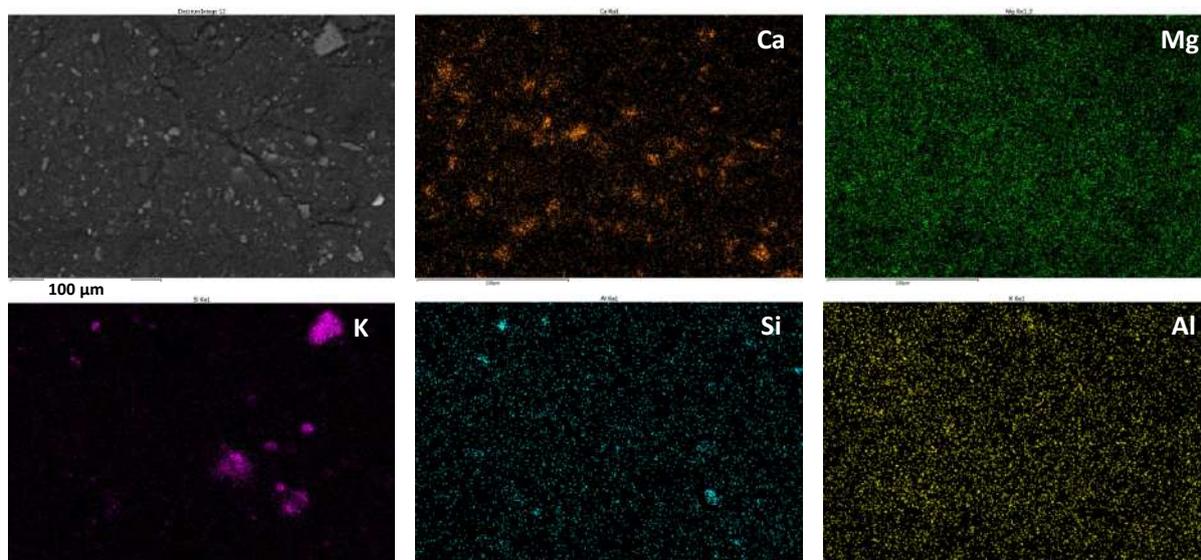


Figure S2. EDS maps obtained for the surface of the BM reference sample.

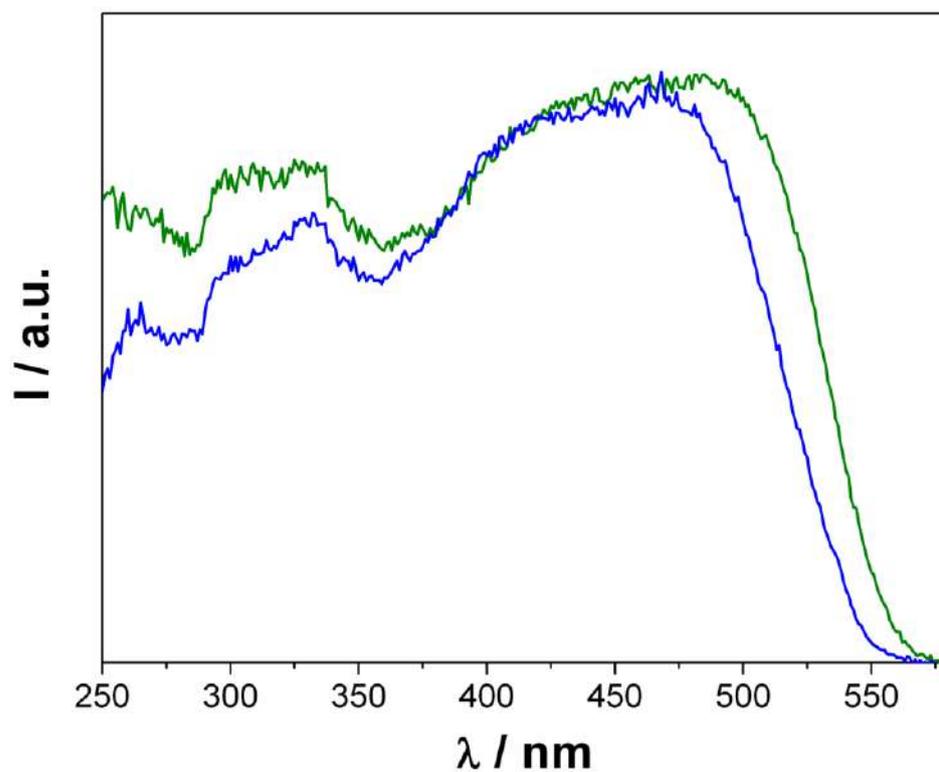


Figure S3. Normalised excitation spectra of NG (olive) and BM (blue), $\lambda_{\text{em}} = 560 \text{ nm}$.

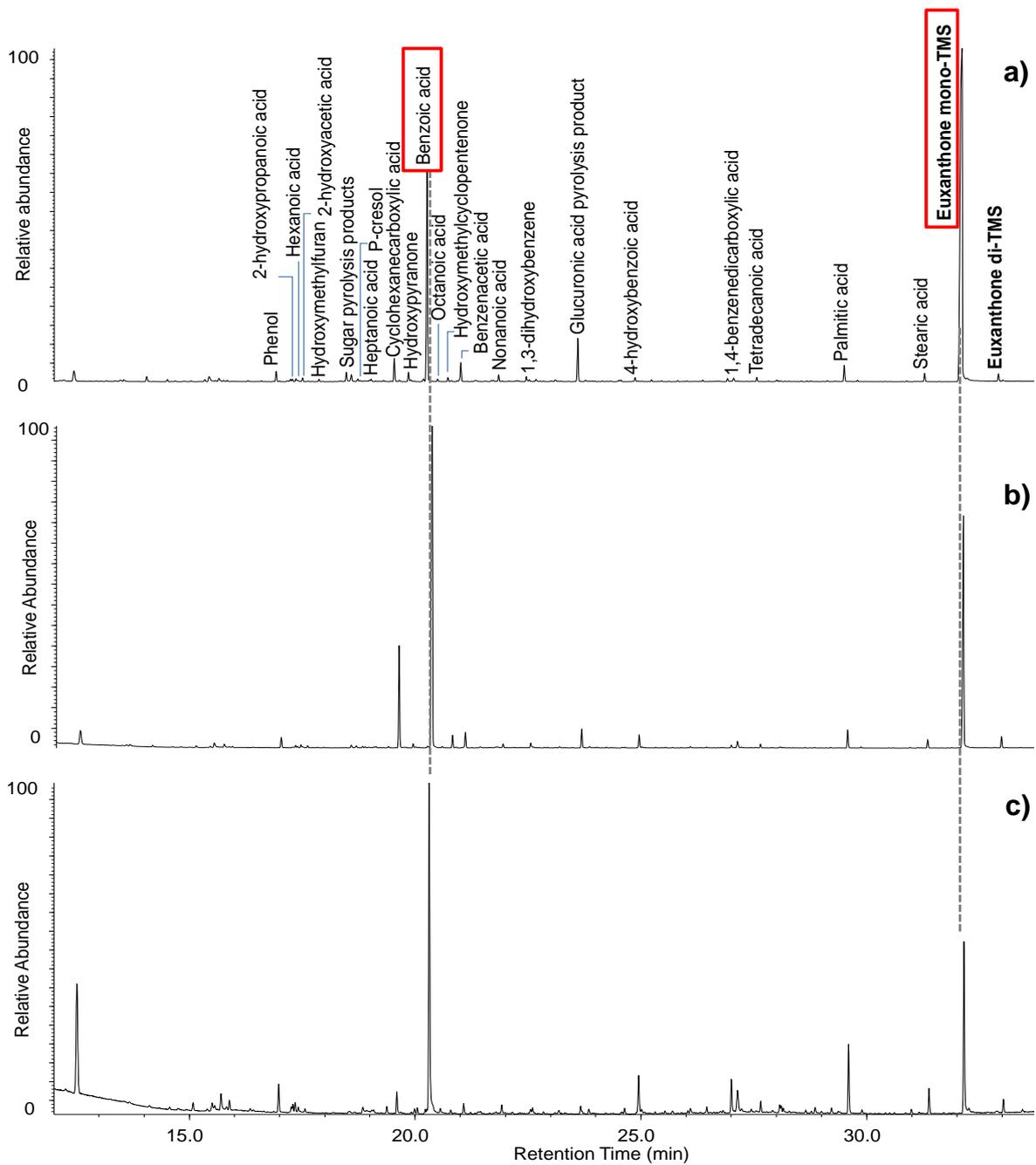


Figure S4. Total Ion Chromatograms (TICs) obtained by Py(HMD)-GC-MS analysis of the NG reference sample of Indian yellow obtained using pyrolysis temperatures **a)** 400, **b)** 500 and **c)** 600°C

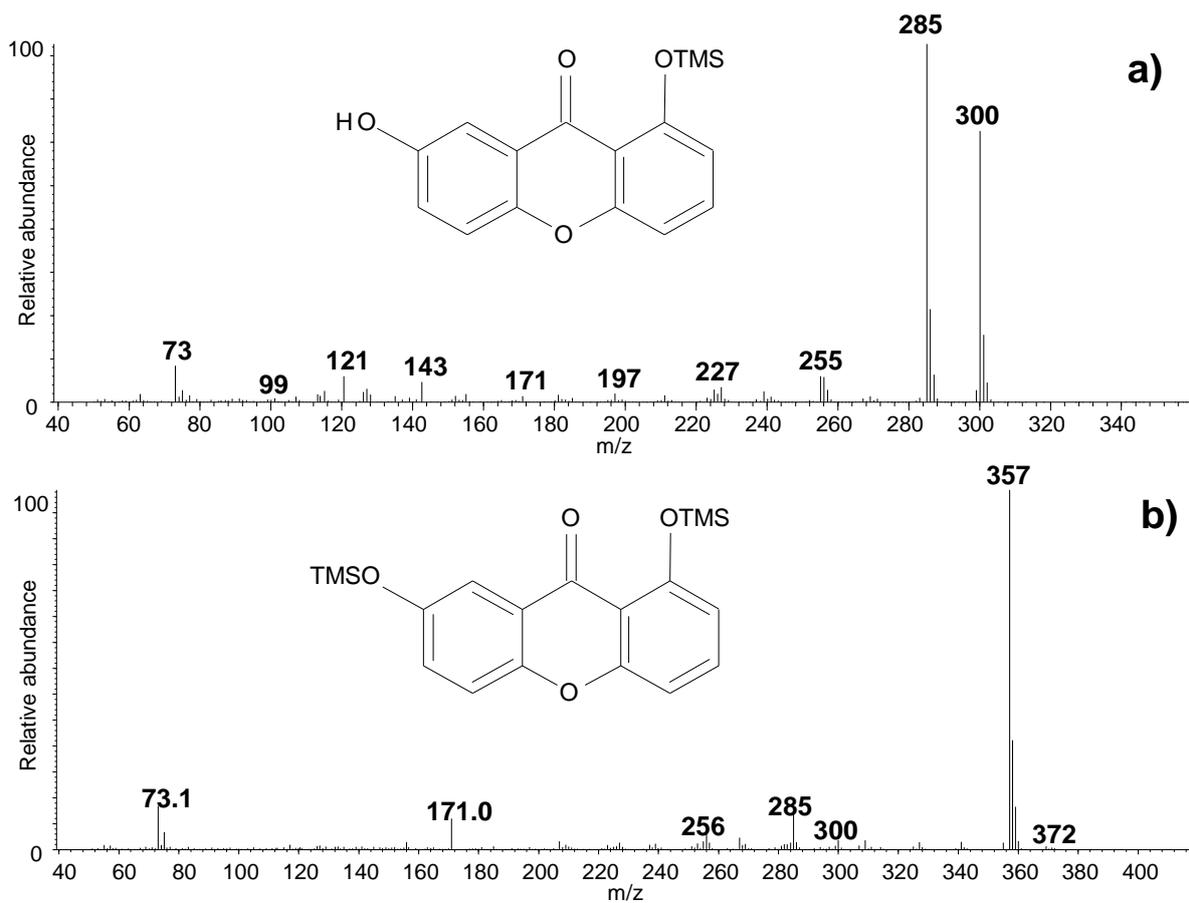


Figure S5. Mass spectra (EI) of **a)** mono-TMS and **b)** bis-TMS euxanthone.