Supplementary Materials

Fast direct detection of natural dyes in historic and prehistoric textiles by Flowprobe™-ESI-HRMS

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Characterization of dyestuff isomers by HPLC-DAD and LC-ESI-triple quadrupole MS/MS (for experimental details, see ref. [1])

Besides carminic acid two other substances were detected in extracts of B and C as well as in extracts of the scale insect *Dactylopius coccus* Costa by LC-ESI-MS/MS, characterized by the presence of the quasi-molecular ion at *m/z* 491 ($[M-H]^-$) and by fragment ions at *m/z* 447, 357 and 327 analogously to carminic acid. Most likely these compounds were isomers of the carminic acid [LC-ESI-MS/MS chromatogram: t_R (carminic acid) = 9.8 min; t_R (isomer 1 of carminic acid) = 10.6 min, t_R (isomer 2 of carminic acid) = 10.9 min, experimental conditions [1]]. The isolation and characterisation of isomers of carminic acid from *Dactylopius coccus* Costa has already been reported by Stathopoulou et al. and Lech et al.[2, 3]. The isomers, known as dcIV (7-C-α-D-glucofuranoside of kermesic acid) and dcVII (7-C-β-D-glucofuranoside of kermesic acid), differ from carminic acid (7-C-α-D-glucopyranoside) in their sugar moiety and are minor constituents of the cochineal dye. Other minor constituents of the cochineal dye are dcII (7-C-α-D-glucopyranoside of flavokermesic acid) and dc1 (C-glucoside and isomer of dcII), characterized by the quasi-molecular ion at *m/z* 475 ($[M-H]^-$) and by fragment ions at *m/z* 431, 341, 311. Extracts of the red-shaded samples B and C showed the same fragmentation pattern.

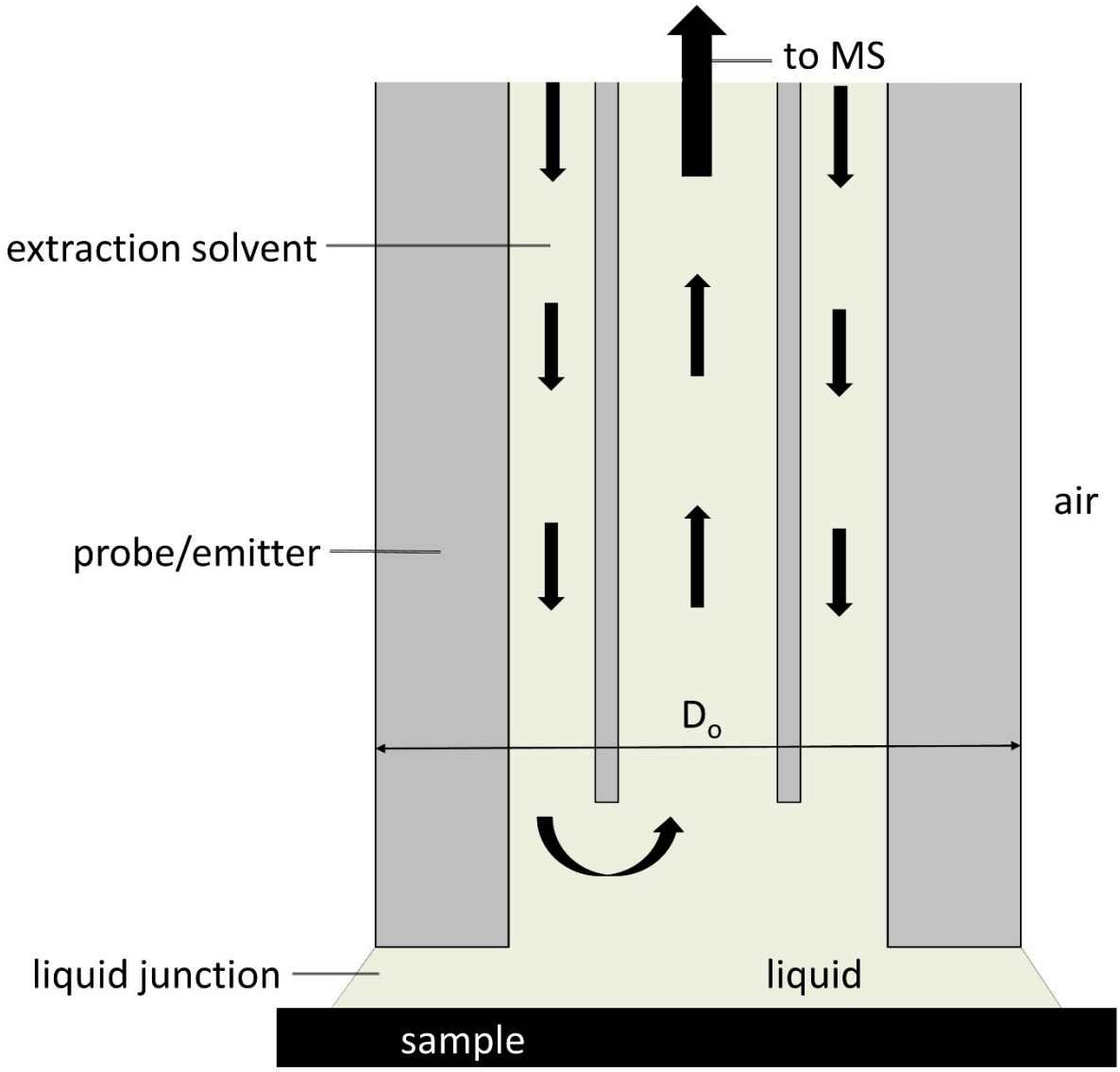

Fig. S1. Schematic illustration of a junction region from a flowprobe™-MS experimental setup with D_o = 630 µm (D_o: probe outer diameter).

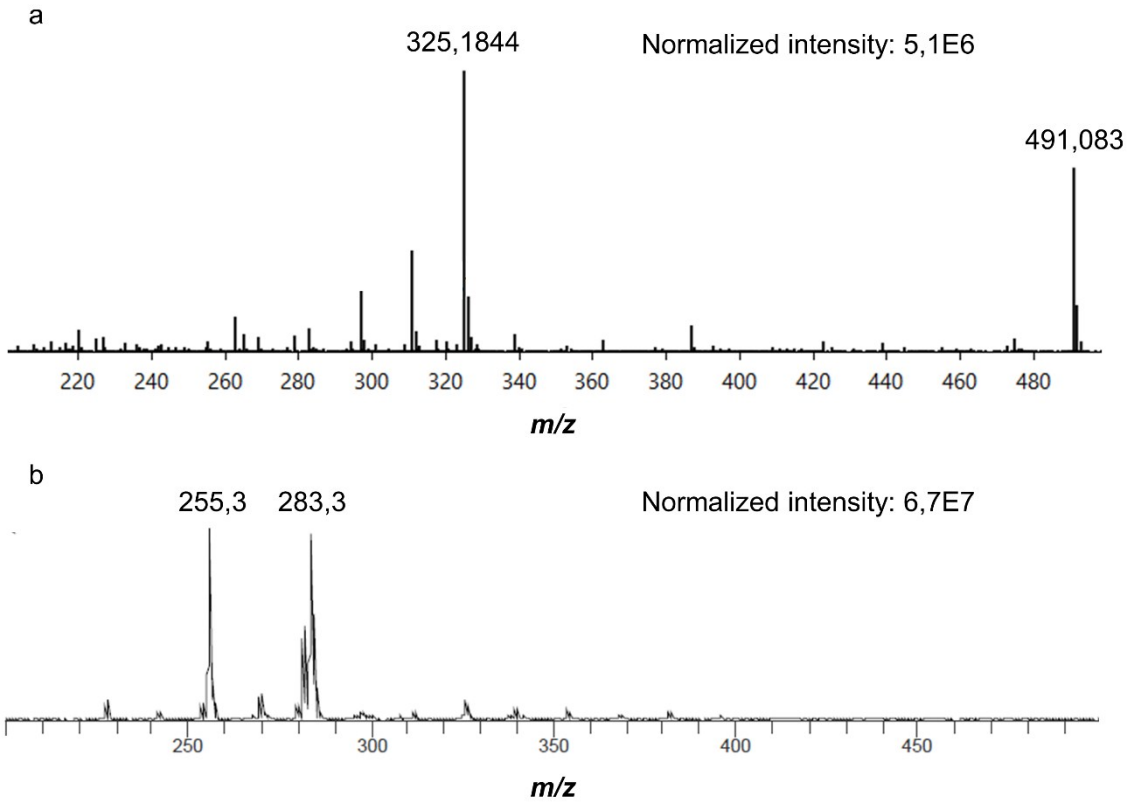

Fig. S2. (a) Flowprobe™ HRMS and (b) ASAP®-MS spectra (negative ion full-scan mode) of woolen reference fibers dyed with an extract of the scale insect *Dactylopius coccus* Costa (main constituent: carminic acid, $[M-H]^-$ at *m/z* 491.083).

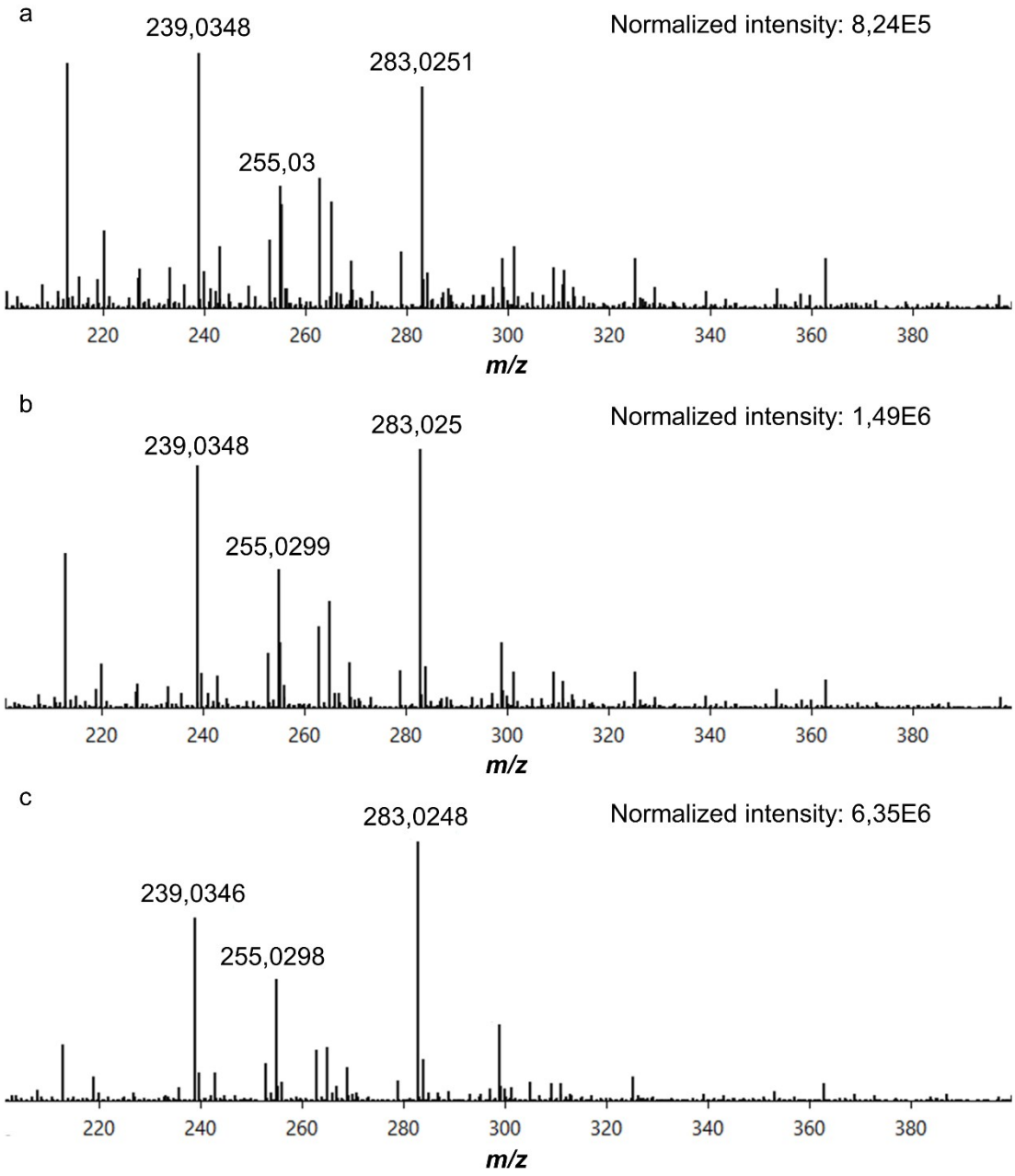

Fig. S3. Flowprobe™ HRMS spectra of silk material dyed with madder after (a) 0.6 min, (b) 1 min and (c) 2.2 min acquisition time (negative ion full-scan mode).

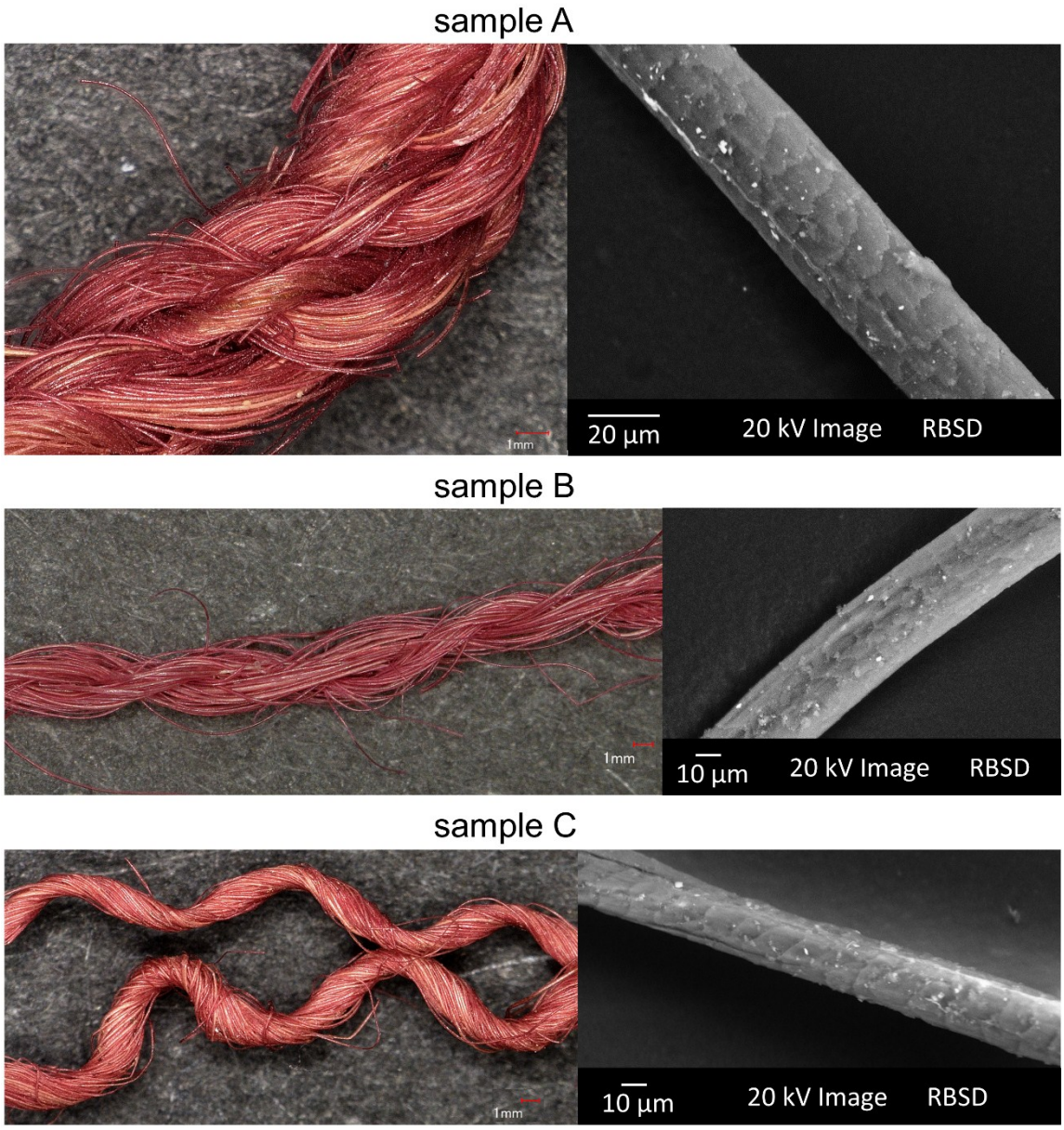

Fig. S4. OM (left) and SEM (right) images of historic sample A (wool), B (wool) and C (wool).

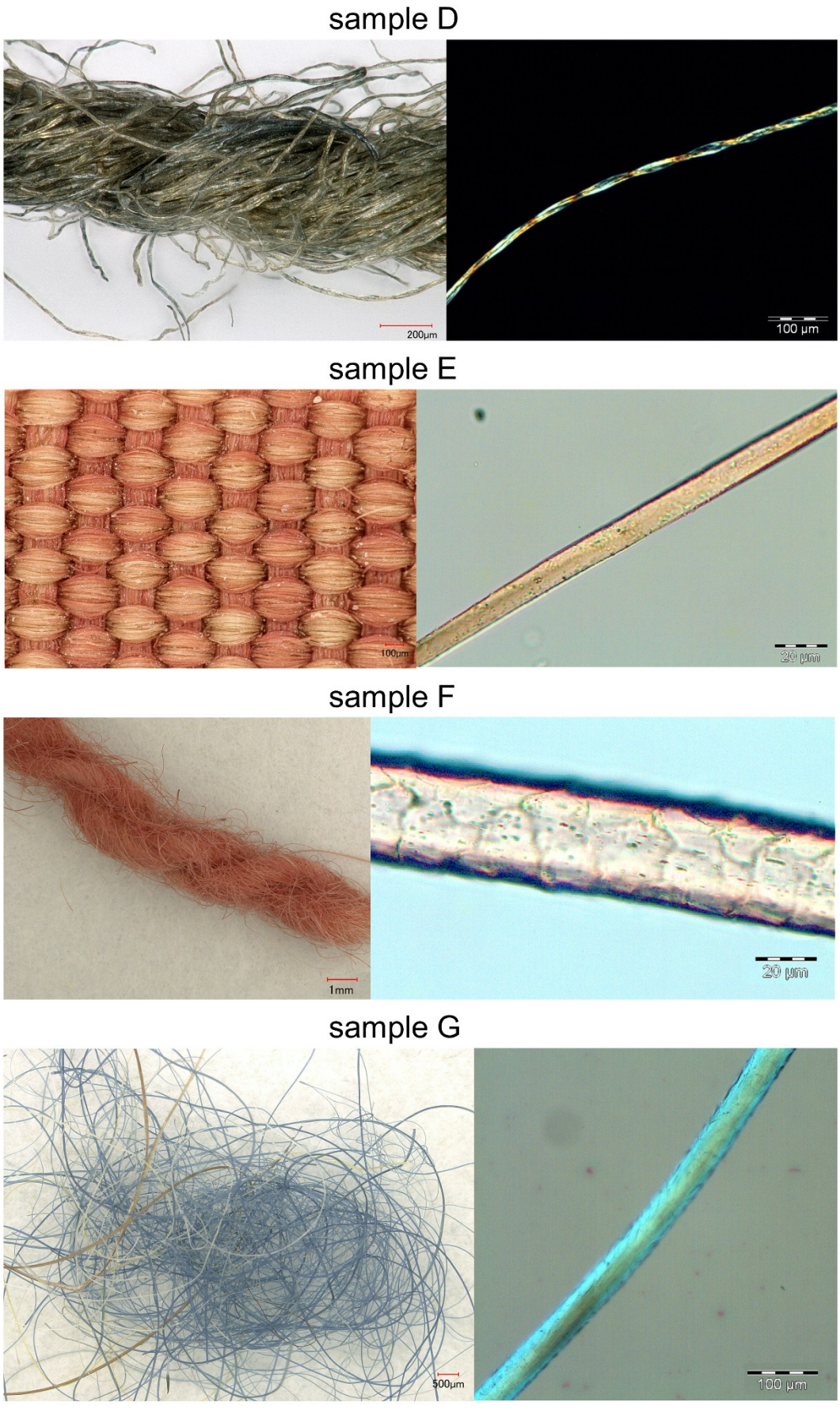

Fig. S5. OM images of historic sample D (cotton), E (silk), F (wool) and G (wool).

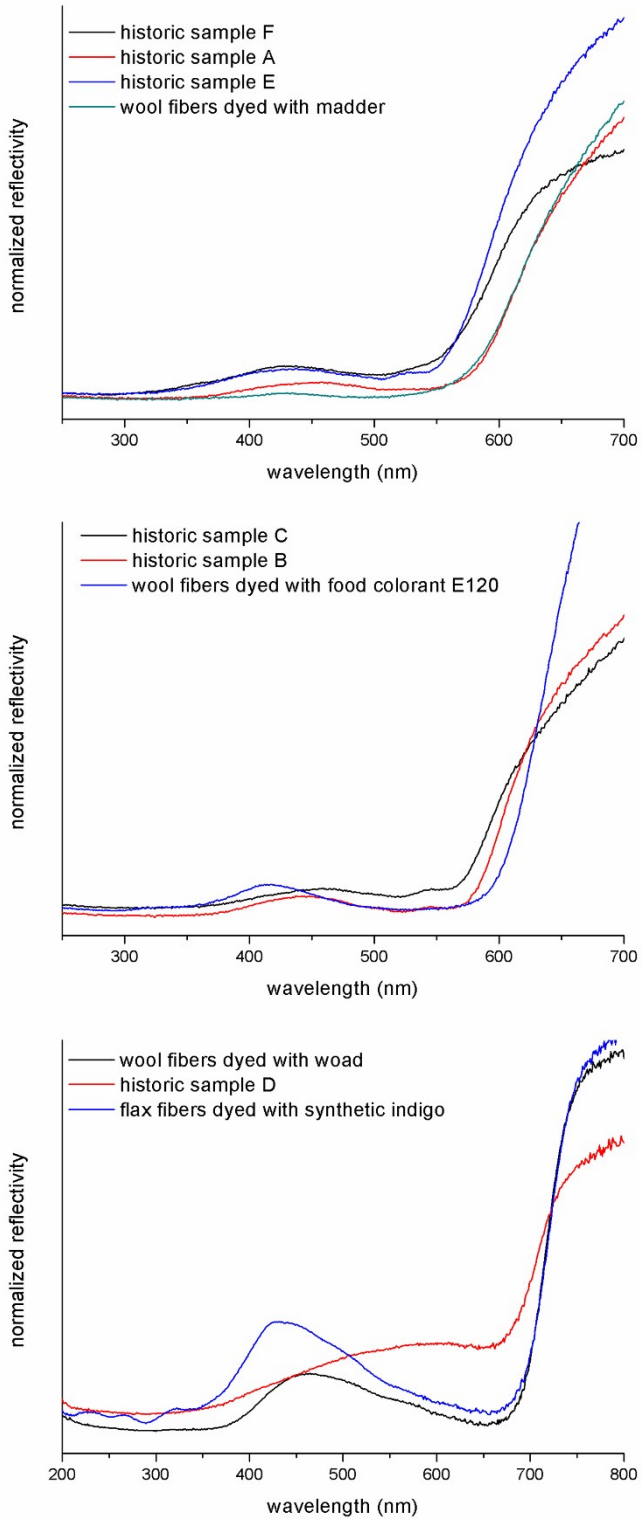

Fig. S6. UV-Vis diffuse reflectance spectra of historic samples and reference materials (wool fibers dyed with madder, food colorant E120 or woad and flax fibers dyed with synthetic indigo).

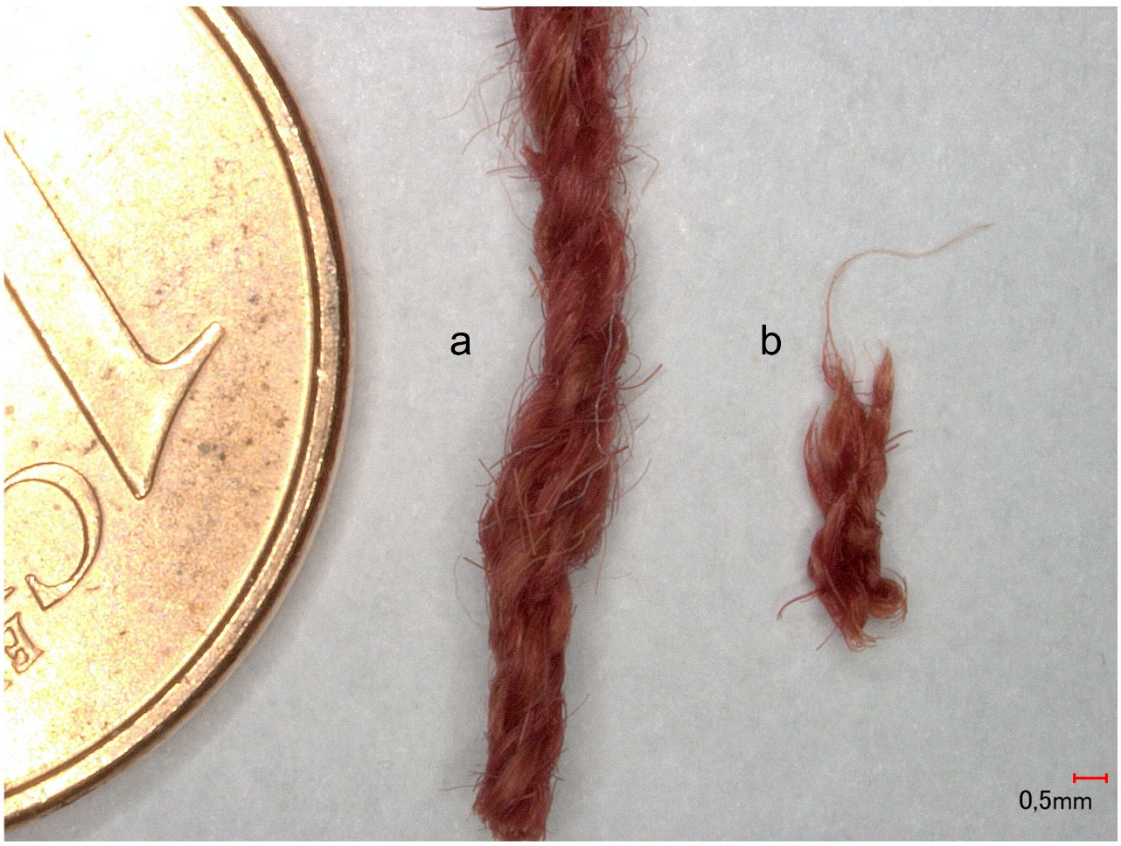

Fig. S7. Image of historic sample A: (a) material prior flowprobe™ MS experiments and (b) aliquot (700 µg) used for analysis by flowprobe™ MS (after measurement).

Tab. S1. MS data of organic dyestuffs detected in the historic samples A-G by flowprobe™ HRMS, errors (ppm) between calculated and measured *m/z* values.

Substances	**Detected ions**	***m/z* values**		**Error (ppm)**
		calculated	**measured**	
C-glucosides of flavokermesic acid	$[C_{22}H_{19}O_{12}]^-$ **[M-H]$^-$**	475.0882	Sample B: 475.0881	0.2
C-glucosides of kermesic acid	$[C_{22}H_{19}O_{13}]^-$ **[M-H]$^-$**	491.0831	Sample B: 491.0832 Sample C: 491.0837	0.2 1.2
Dihydroxyanthraquinones	$[C_{14}H_7O_4]^-$ **[M-H]$^-$**	239.0350	Sample A: 239.0348 Sample E: 239.0347 Sample F: 239.0349	0.8 1.2 0.4
Dihydroxyanthraquinone-carboxylic acids	$[C_{15}H_7O_6]^-$ **[M-H]$^-$**	283.0248	Sample A: 283.0250 Sample E: 283.0249 Sample F: 283.0252	0.7 0.4 1.4
Dihydroxy-methylanthraquinones	$[C_{15}H_9O_4]^-$ **[M-H]$^-$**	253.0506	Sample A: 253.0506 Sample C: 253.0506 Sample E: 253.0505 Sample F: 253.0508	0 0 0.3 0.7
Flavokermesic acid	$[C_{16}H_9O_7]^-$ **[M-H]$^-$**	313.0354	Sample B: 313.0355 Sample C: 313.0358	0.3 1.3
Indigo/indirubin	$[C_{16}H_{11}N_2O_2]^+$ **[M+H]$^+$**	263.0815	Sample D: 263.0819 Sample G: 263.0818	1.5 1.1
Kermesic acid	$[C_{16}H_9O_8]^-$ **[M-H]$^-$**	329.0303	Sample B: 329.0303 Sample C: 329.0306	0 0.9
Trihydroxyanthraquinones	$[C_{14}H_7O_5]^-$ **[M-H]$^-$**	255.0298	Sample A: 255.0299 Sample C: 255.0299 Sample E: 255.0298 Sample F: 255.0301	0.4 0.4 0 1.2

References

1. A. Kramell, X. Li, R. Csuk, M. Wagner, T. Goslar, P. E. Tarasov, N. Kreusel, R. Kluge and C.-H. Wunderlich, *Quat. Int.*, 2014, **348**, 214-223.
2. K. Lech, K. Witkoś, B. Wileńska and M. Jarosz, *Anal. Bioanal. Chem.*, 2015, **407**, 855-867.
3. K. Stathopoulou, L. Valianou, A.-L. Skaltsounis, I. Karapanagiotis and P. Magiatis, *Anal. Chim. Acta*, 2013, **804**, 264-272.