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Investigation of natural dyes occurring in historical Coptic textiles by high-performance liquid chromatography with UV–Vis and mass spectrometric detection

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Abstract

Liquid chromatography (LC) combined with ultraviolet–visible (UV–Vis) and mass spectrometric (MS) detection was utilized to study the chemical components present in extracts of natural dyes originating from fiber samples obtained from Coptic textiles from Early Christian Art Collection of National Museum in Warsaw. Chromatographic retention, ionization, UV–Vis and mass spectra of twenty selected dye compounds of flavanoid-, anthraquinone- and indigo-types were studied. Most of the investigated compounds could be ionized by positive and negative ion electrospray ionization. Difficulties with the ionization by electrospray were experienced for indigotin and brominated indigotins, but these were ionized by atmospheric pressure chemical ionization. Mass spectrometric detection, utilizing different scanning modes of a triple quadrupole mass spectrometer, combined with the UV–Vis detection was demonstrated to be a powerful approach to detection and identification of dyes in the extracts of archeological textiles. Using this approach the following compounds were identified in the extracts of Coptic textiles: luteolin, apigenin, rhamnetin, kaempferol, alizarin, purpurin, xanthopurpurin, monochloroalizarin, indirubin, and so the type of dye that was utilized to dye the textiles could be identified. Detection capabilities for several dye-type analytes were compared for the UV–Vis and mass spectrometric detection. The signal-to-noise ratios obtained for luteolin, apigenin, and rhamnetin were higher for the MS detection for most of the examined sample extracts. Purpurin, alizarin, and indirubin showed similar signal-to-noise ratios for UV–Vis and mass spectrometric detection.

Keywords: Textiles; Art analysis; Dyes

1. Introduction

Analytical separation techniques combined with sensitive and selective detection techniques, play an increasing role in the chemical investigation of archeological objects. The knowledge derived from the chemical composition of materials utilized for dying archeological objects not only assists sometimes in their dating and locating their origins but also provides invaluable insights to the application of appropriate treatments during conservation and resto-

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ration work. Historically, thin-layer chromatography (TLC) has often been employed to separate the chemical components of natural dyes, but the detection, identification, and quantitative spot evaluation limit its usefulness. These limitations are significantly alleviated by application of high-performance liquid chromatography (HPLC) with UV–Vis detection. Wouters [1], in his pioneering work on historical textiles, utilized a reversed-phase HPLC separation with formic acid in a water–methanol mobile phase and UV–Vis detection for analysis of plant-root and insect extracts of red dyestuffs. The full capabilities of UV–Vis detection were employed by Wouters and Verhecken [2] using the full-range UV–Vis spectra to aid the identification of red dyes of insect origin. HPLC–DAD (diode array detection) measurements were also employed for investigation of dyes in historical objects by Nowik [3], and to examine some natural and synthetic dyes by Fischer et al. [4]. Red natural dyes of cochineal, lac, kermes, and madder were analyzed by HPLC–DAD by Hayashi and Masako [5] allowing identification of carminic acid, laccaic acid, kermesic acid, alizarin, purpurin, and pseudopurpurin. Koren [6] studied plant and insect red dyes, molluscan blue, and red-purple indigoid vat dyes, the dyes most often found in ancient textiles and shards by HPLC. Wouters [7] analyzed dyes present in Coptic textiles of the 3rd–10th centuries in Belgian private collections and natural dyes in a textile from the Lennoxlove toilet service [8] by HPLC–DAD.

Liquid chromatography–mass spectrometry (LC–MS) has rarely been used to study dyes of ancient textiles. LC–MS with thermospray ionization and selected ion monitoring was applied to investigate red dyes of woven fabrics from the Greco–Roman period by Yamaoka et al. [9], allowing detection and identification of alizarin. Ferreira et al. [10] utilized electrospray ionization (ESI) on a quadrupole ion trap mass spectrometer to study flavanoid-type natural yellow dyes by direct infusion of the extract obtained from wool dyed with weld or onion skin dyes. Recently, Ferreira et al. [11] used LC–ion trap mass spectrometry with electrospray ionization to study photodegradation products of wool dyed with yellow natural dyes. The extracts of indigoid natural dyes derived from *Murex* molluscs were investigated by Michel et al. [12] by high-resolution MS.

Extraction of dyes from a textile fiber is usually carried out with 3 *M* hydrochloric acid–methanol (1:1, v/v) at the boiling point. This procedure allows efficient extraction of anthraquinone and flavanoids dyes from textile fibers and hydrolysis of their glycosidic forms to aglycones [13,14]. This procedure is inefficient for extraction of indigotin and indirubin, but small amounts of indigotin can be extracted this way [15]. 6,6′-Dibromoindigotin, the main component of real purple is not soluble under such conditions; hence for blue or purple fibers the extraction with warm pyridine [16], dimethylsufoxide (DMSO) [12], or dimethylformamide (DMF) [17] is recommended.

The aim of this study was the detection and identification of chemical components present in extracts of natural dyes in the samples of different color fibers taken from Coptic textiles from Early Christian Art Collection in the National Museum in Warsaw [18]. LC–MS, utilizing different scanning modes of a triple quadrupole mass spectrometer and UV–Vis detection were employed to achieve that goal. The secondary goal was the comparison of the strengths and drawbacks of the two detection techniques in a situation of limited sample availability and a need for broad characterization of a complex extract sample.

2. Experimental

2.1. Chemicals

The reference substances were either purchased from commercial sources or obtained as gifts from individual researchers. Alizarin, purpurin, carminic acid, ellagic acid (>98%), gallic acid (>98%), apigenin (>95%) were obtained from Fluka (Buchs, Switzerland). The following reference substances were obtained from the different sources: luteolin, rhamnetin, kaempferol, and quercetin (Roth, Karlsruhe, Germany), lawsone, and myricetin (>85%) (Sigma, Steinheim, Germany), indigotin and lac dye (Kremer, Krakow, Poland). Isoindirubin, 6-monobromoindigotin, and 6,6′-dibromoindigotin were kindly made available by Chris J. Cooksey (Watford, UK).

The solvents used for chromatographic separations

or standards preparation (methanol, water, acetonitrile) were HPLC grade, obtained from EM Science (Merck, Darmstadt, Germany). Formic acid (>98%) was obtained from Riedel-de Haen (Seelze, Germany). The reagents used for sample extraction were ethanol (95%) (Polmos, Poland), hydrochloric acid (25%) (Merck), and deionized water.

Samples of fibers were obtained from different color Coptic textiles from Early Christian Art Collection of National Museum in Warsaw. The collection comprises of eighty Coptic textiles supposed from the 4th to the 12th century. Different articles of clothing of everyday use of that era, fragments of decorative girdles, panels, appliqués, and a small conical cap belong to this collection. Samples of fibers about 1 cm long of the selected articles were taken for chemical investigation of the natural dyes used for dyeing them.

2.2. Apparatus

Two different LC–MS systems were employed. Initially, the work was started on a triple quadrupole mass spectrometer Quattro LC (Micromass, Manchester, UK) equipped with a Z-spray API (atmospheric pressure ionization) ESI ion source, interfaced with HP 1100 HPLC system (Agilent, Palo Alto, CA, USA) and in-line variable-wavelength ultraviolet detector (Agilent) (System I). The work was continued on a triple quadrupole mass spectrometer Quattro Micro (Micromass) equipped with Z-spray API ESI ion source or APCI (atmospheric pressure chemical ionization) ion source, combined with a 2795 Waters HPLC system (Waters, Milford, MA, USA) and in-line Model 2996 PDA DAD UV–Vis detector (Waters) (System II). All separations were carried out using the Zorbax RX-C_{18}, 150×2.1 mm, 5 μm particle column (Agilent). The mobile phase consisted of 0.3% (v/v) formic acid in water (A) and acetonitrile (B). The following gradient was employed for standards and sample analysis: 5% B (isocratic) to 2 min, to 60% B (linear) at 30 min, to 100% B (linear) at 35 min, 100% B (isocratic) to 60 min at the flow-rate of 0.2 ml/min. However, the same gradient resulted in different retention times for Systems I and II [e.g. luteolin eluted at 19.2 min with System II and 25.0 min with System I, apigenin at 21.5 min (System II) and 27.2 min (System I)]. That constant shift of the retention times between systems can be attributed to different values of the gradient delay time, specific to the LC systems employed. The column oven temperature in System I was 30 °C and 20 °C in System II.

The ESI probe and ion source were operated at 3.5 kV of capillary voltage, cone voltage: 30 V, source temperature: 120 °C, desolvation temperature: 300 °C, cone gas flow-rate: 60 l/h, and desolvation gas flow-rate: 500 l/h for both negative and positive ion generation with System II. The APCI probe and ion source were operated at corona current of 2 μA, cone voltage: 50 V, source temperature: 130 °C, desolvation temperature: 500 °C, cone gas flow-rate: 100 l/h, and desolvation gas flow-rate: 300 l/h for System II. The ESI and ion source of System I were operated at 3.0 kV of capillary voltage for negative ions and 3.5 kV for positive ions, cone voltage: 30 V, source temperature: 120 °C, desolvation temperature: 300 °C, nebulizer gas flow-rate: 150 l/h, and drying gas flow-rate: 700 l/h.

2.3. Sample preparation and experimental procedures

Solutions of the reference substances were initially prepared in water–methanol (50:50) at approximately 100–500 μg/ml and then diluted 1:10 with methanol. Several of the reference substances did not completely dissolve under these conditions. Therefore, the solutions were filtered using a nylon syringe filter and saturated solutions of these reference substances were used. Taking into account the qualitative nature of this work, no attempt was made to change the solvent in order to completely dissolve these reference substances.

The solutions of the reference substances were co-infused to the mass spectrometer at 50 μl/min using a syringe pump with acetonitrile and 0.3% (v/v) formic acid in water (50:50) mobile phase flown from the HPLC system at 0.2 ml/min. All data for the reference substances were obtained with System II. Negative and positive electrospray ionization was examined for each of the reference substances. The reference substances that did not ionize by electrospray were subjected to APCI. The tuning parameters were not fully optimized to obtain the maximum sensitivity for the individual reference

substances. The values of tuning parameters described above were used. The MS spectrum and CID spectra at different collision energies (20, 30 and 40 eV) were recorded for each reference substance. The chromatographic performance of the reference substances was evaluated by injection of 20-μl aliquots of the reference substance solutions into System II. The UV–Vis spectra in the range 200–700 nm and the MS scan spectra from 100 to 700 m/z were collected for the entire chromatographic run (60 min) for each reference substance.

The extracts of the authentic fiber samples of Coptic textiles were prepared by an open tube hydrolysis of 0.5–3 mg of the fiber in 3 *M* hydrochloric acid–ethanol (1:1, 400 μl) at 100 °C for 20 min. The remaining extract was filtered using a polypropylene centrifuge filter (VectaSpin Micro, 0.45 μm, Whatman, Maidstone, UK) and then evaporated to dryness in a vacuum desiccator. The residue was dissolved in 0.5 ml of water–methanol (50:50) before analysis. Typically, 50 μl of the extract was injected into the LC–MS system.

The extracts of the fiber samples were analyzed by HPLC combined with UV–Vis and MS detection. The HPLC–UV–MS analysis was performed utilizing the scanning options of a triple quadrupole mass spectrometer. The initial runs were geared towards identifying the largest number of potential chemical species in the extracts. Therefore, the chromatographic runs were done utilizing MS scan mode (100–700 m/z) throughout the whole run and UV detection at 255 nm on the System I. Separate runs were done for each sample using the positive and negative electrospray ionization. Based on the full scan MS data and UV peaks, the pseudomolecular ions of the prominent peaks were determined and another series of chromatographic runs was set up with the mass spectrometer acquiring the daughter ion spectra of selected ions around the retention time of the selected ion. The samples were also run on System II using negative ion ESI, MS scan mode (100–700 m/z), and UV–Vis scan (200–700 nm) through the entire chromatographic run. The MS and UV–Vis data from both systems allowed identification of a set of chemicals that were of interest as components of dyeing mixtures. In order to enhance the detectability, the mass spectrometer was set up in the MS–MS mode (referred to as MRM on the Micromass systems) to monitor characteristic ion transitions for selected species. The selected transition were the pseudomolecular ion on MS1 and the base peak of the daughter ion spectrum on the MS2.

3. Results and discussion

3.1. Chromatographic, mass spectrometric, and UV–Vis properties of reference substances

Chromatographic retention times, UV–Vis spectrum major maxima, mass to charge ratios (m/z) for obtained pseudomolecular ions, and the appearance of the CID spectrum of the pseudomolecular ion for all twenty investigated reference substances are summarized in Table 1. The ions exhibiting generally more than 10% of base peak intensity are listed in Table 1 for the CID spectra.

Flavanoids have recently been the focus of many MS investigations because the ability of the HPLC–MS techniques to identify and selectively quantify them in complex matrices of plant and food extracts [19–21]. The CID spectra of selected flavanoids obtained with negative ion electrospray and APCI ionization and their chromatographic separations were examined by Hughes et al. [22] and Justesen [23], respectively. The fragmentation mechanisms of flavanoids were described in detail by Ma and coworkers [24,25] for positive, protonated molecular ions and by Fabre et al. [26] for negative, deprotonated molecular ions. The negative ion CID spectra obtained in this study for the examined flavanoids agree with those reported in the literature, when similar instrumentation is used. The CID spectra reported by Hughes et al. [22] for apigenin and quercetin obtained on Quattro LC triple quadrupole MS agree with these obtained in this work. However, the CID spectra of flavanoids reported by Fabre et al. [26], obtained on the LCQ ion trap MS are much richer in ions of larger mass. Similarly, the CID spectra for positive ions obtained by Ma et al. [24] on the VG 70-SEQ mass spectrometer correlate relatively well with these presented in Table 1. This comparison stresses the importance of collecting the CID spectra of the reference substances on the particular instrumentation in order to use them for further identification of unknowns. The fragmenta-

Table 1
Summary of collision induced dissociation (CID) spectra, pseudomolecular ions, UV–Vis maxima, and retention times (t_R) obtained for examined reference substances

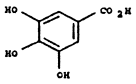
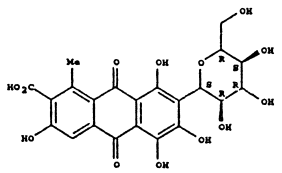
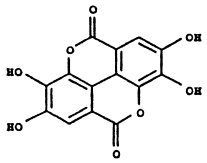
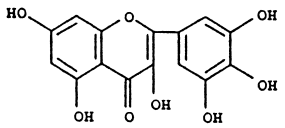
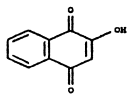
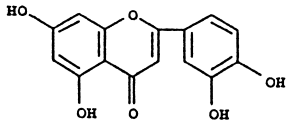
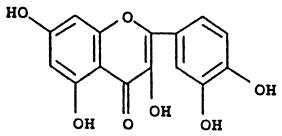
Name	Structure	t_R (min)	UV–Vis max (nm)	Ionization mode	$[M-H]^-$ or $[M+H]^+$ (m/z)	Major CID fragment ions: m/z (%BPI[a])	Other CID fragment ions: m/z (%BPI)
Gallic acid		3.0	220, 271	ESI−[b]	169	79 (100), 69 (39), 124 (27)	125 (13), 97 (7), 95 (6), 81 (10), 67 (13), 55 (7), 53 (18)
Carminic acid		12.8	275, 222, 493	ESI−	491	327 (100), 357 (83), 299 (54)	447 (8), 369 (7), 339 (13), 299 (54), 298 (38), 285 (8)
				ESI+[c]	493	355 (100), 325 (23), 337 (22)	403 (14), 391 (11), 379 (18), 373 (5), 351 (5)
Ellagic acid		14.0	253, 366	ESI−	301	145 (100), 284 (70), 185 (68)	301 (77), 300 (54), 257 (6), 245 (17), 229 (36), 201 (49), 173 (59), 157 (34), 129 (44), 117 (21), 101 (15)
Myricetin		16.7	368, 253	ESI−	317	151(100), 137 (84), 107 (41)	317 (9), 179 (19), 165 (7), 125 (5), 109 (27), 83 (18), 65 (22), 63 (18)
				ESI+	319	153 (100), 111 (33), 165 (27)	319 (49), 273 (14), 245 (21), 217 (22), 137 (21), 109 (16), 69 (29)
Lawsone		17.2	245, 279,338	ESI−	173	77 (100), 145 (90), 101 (36)	–
Luteolin		19.3	348, 253	ESI−	285	133 (100), 107 (24), 151 (22)	285 (5), 175 (10), 149 (9), 83 (8), 65 (10)
				ESI+	287	153 (100), 135 (42), 117 (16)	287 (61), 241 (8), 171 (10), 161 (13), 89 (14)
Quercetin		19.5	368, 255	ESI−	301	107 (100), 151 (99), 121 (70)	164 (7), 163 (6), 149 (9), 93 (16), 83 (44), 65 (61)
				ESI+	303	153 (100), 137 (81), 229 (58)	303 (28), 285 (7), 257 (19), 201 (41), 165 (31), 121 (22), 109 (23), 69 (27)

Table 1. Continued

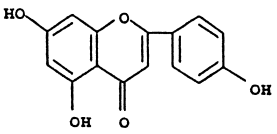
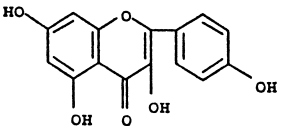
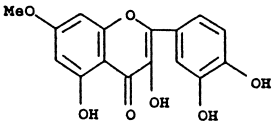
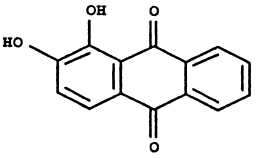
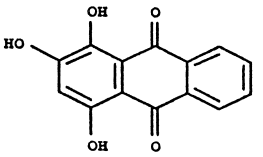
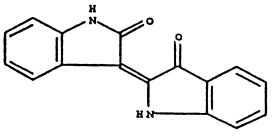
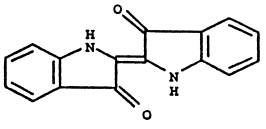
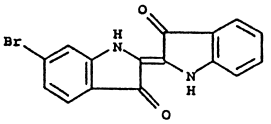
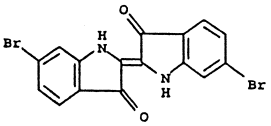
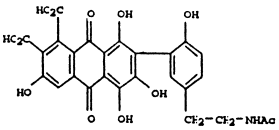
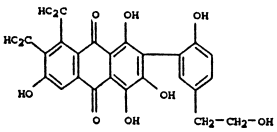
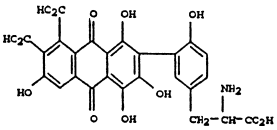
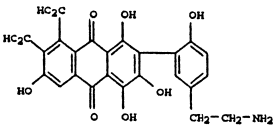
Name	Structure	t_R (min)	UV–Vis max (nm)	Ionization mode	$[M-H]^-$ or $[M+H]^+$ (m/z)	Major CID fragment ions: m/z (%BPI[a])	Other CID fragment ions: m/z (%BPI)
Apigenin		21.5	337, 267	ESI−	269	117 (100), 107 (33), 151 (18)	159 (6), 149 (15), 121 (8), 83 (12), 65 (21)
				ESI+	271	153 (100), 119 (59), 91 (27)	271 (56), 171 (5), 163 (8), 145 (14), 121 (20)
Kaempferol		22.0	364, 265	ESI−	285	93 (100), 107 (58), 159 (48)	285 (70), 239 (23), 211 (27), 187 (37), 185 (47), 145 (38), 143 (33), 119 (29), 117 (35)
				ESI+	287	153 (100), 121 (59), 165 (32)	287 (37), 258 (9), 213 (20), 185 (8), 171 (9), 137 (23), 107 (22), 69 (27)
Rhamnetin		24.7	368, 255	ESI−	315	121 (100), 165 (94), 97 (44)	163 (6), 151 (13), 149 (8), 107 (10), 93 (17), 65 (45)
				ESI+	317	167 (100), 123 (85), 274 (70)	317 (72), 302 (29), 274 (70), 243 (52) 215 (42), 179 (32), 137 (60), 109 (16)
Alizarin		25.3	248, 427	ESI−	239	210 (100), 211 (51), 167 (34)	239 (38), 238 (11), 183 (9), 155 (22), 127 (17), 101 (5)
Purpurin		28.1	256, 294, 480	ESI−	255	129 (100), 227 (85), 171 (55)	255 (17), 226 (13), 183 (27), 159 (12), 157 (24), 143 (21), 101 (19)
				ESI+	257	187 (100), 159 (86), 131 (77)	257 (18), 229 (58), 183 (21), 155 (37), 145 (19), 117 (28), 115 (23), 111 (21)
		29.8	290, 542, 362	ESI+	263	219 (100), 190 (72), 235 (28)	263 (16), 245 (13), 217 (35), 206 (22), 180 (13), 165 (22), 132 (15)
Indirubin							
Indigotin				APCI+[d]	263	77 (100), 132 (71), 104 (37)	263 (40), 262 (20), 235 (27), 234 (26), 219 (30), 206 (37), 190 (16), 180 (12), 116 (12)

Table 1. Continued

Name	Structure	t_R (min)	UV–Vis max (nm)	Ionization mode	$[M-H]^-$ or $[M+H]^+$ (m/z)	Major CID fragment ions: m/z (%BPI[a])	Other CID fragment ions: m/z (%BPI)
6-Bromoindigotin				APCI+	341	205 (100), 262 (76), 218 (38)	341 (71), 313 (32), 297 (23), 286 (10), 234 (25), 158 (18), 131 (22), 77 (31)
6,6′-Dibromo-indigotin				APCI+	419	340 (100), 312 (9), 375 (7)	419 (21), 418 (7), 234 (6), 205 (9)
Laccaic acid A		14.7	287, 225, 490[e]	ESI−	536	430 (100), 448 (54), 474 (10)	492 (7), 358 (6)
Laccaic acid B		14.5	287, 225, 490[e]	ESI−	495	389 (100), 407 (29), 358 (8)	451 (5), 433 (6)
Laccaic acid C		11.7	287, 225, 490[e]	ESI−	538	405 (100), 432 (84), 450 (72)	494 (29), 476 (13), 387 (50), 376 (37), 371 (57)
Laccaic acid E[f]		12.1	287, 225, 485[e]	ESI−	494	405 (100), 388 (99), 371 (87)	494 (19), 474 (11), 465 (12), 450 (60), 432 (72), 406 (51), 358 (39), 348 (16), 318 (47), 229 (32)

[a] % Base peak intensity (% BPI).
[b] Electrospray ionization, negative ions (ESI−).
[c] Electrospray ionization, positive ions (ESI+).
[d] Atmospheric pressure chemical ionization, positive ions (APCI+).
[e] Some of the laccaic acid peaks overlapped. The observed UV–Vis maxima for the minor laccaic acids might be dominated by the spectrum of the major laccaic acid eluting at the same retention time.
[f] Potentially mixed daughter ion spectrum of laccaic acid E and laccaic acid C fragment ion 494 (loss of 44 in-source).

tion patterns of alizarin and purpurin are centered on consecutive losses of CO (mass 28) and CO_2 (mass 44) from the molecule, rather than the middle ring breakage as for the flavanoids. The fragmentation mechanism of alizarin is broadly discussed in the next section.

Indigotin and indirubin demonstrated a somewhat surprising behavior towards ion formation by ESI. The protonated molecular ions of indirubin are relatively easily obtained but indigotin does not ionize. Similarly, two brominated analogs of indigotin, 6-bromoindigotin and 6,6′-dibromoindigotin did not ionize by electrospray. This behavior can be rationalized by the possibility of indigotin forming an internal hydrogen bond between the nitrogen and oxygen and blocking the ability of the molecule to accept a proton from the solution during the ESI. The internal hydrogen bonding is probably sterically hindered in the molecule of indirubin and the molecule is able to accept a proton during the ESI. The ionization of indigotin and its brominated analogs was achieved by APCI in the positive ion mode. Characteristic isotope patterns confirming the presence of one and two bromines were observed for 6-bromoindigotin and 6,6′-dibromoindigotin. The CID spectra of these compounds were obtained in the co-infusion mode for the monoisotopic peaks and are summarized in Table 1. The chromatographic peaks of the indigotin and its brominated analogs were difficult to identify. The LC–MS run with APCI ionization did not show a molecular ion peak for indigotin and brominated analogs. The ESI, positive ion runs with higher concentrations of saturated stock solutions showed a very broad chromatographic peak for indigotin, and no detectable signal for brominated analogs. Therefore, the chromatographic conditions used in this study were not optimal for separation and detection of indigotin and its brominated analogs.

Laccaic acids comprise an important group of naturally occurring dyes. They are usually derived from the lac dye, which is predominately a mixture of laccaic acids. Individual laccaic acids are difficult to obtain, therefore, the commercially available lac dye was used in this work as a source of laccaic acids. Laccaic acid has rarely been studied by MS. Oka et al. [27] used counter-current chromatography for isolation of the laccaic acids from lac and electrospray tandem MS to detect individual acids after HPLC separation. White and Kirby [28] examined laccaic acids derived from lac lakes by the positive ion electrospray MS. The paper discusses fragmentation patterns for positive ions of methylated and underivatized laccaic acids.

The mass spectra obtained for the co-infused lac dye solution for the positive and negative electrospray ion mode are presented in Fig. 1. The CID spectra for all the predominant ions observed in the negative ion spectrum (Fig. 1A) of lac dye were obtained at collision energy of 30 eV. The lac dye solution was also examined by LC–MS with negative ion electrospray and UV–Vis detection. The data indicates the presence of laccaic acid A ($[M-H]^- = 536\ m/z$), laccaic acid B ($[M-H]^- = 495\ m/z$), laccaic acid C ($[M-H]^- = 538\ m/z$), laccaic acid E ($[M-H]^- = 494\ m/z$), and xantholaccaic acid A ($[M-H]^- = 520\ m/z$) in the lac dye solution. This was concluded based on the appearance of positive and negative ion spectra of lac dye (Fig. 1) and chromatographic separation of the lac dye solution. Table 1 contains the retention times of peaks observed for the above laccaic acids. Analysis of the

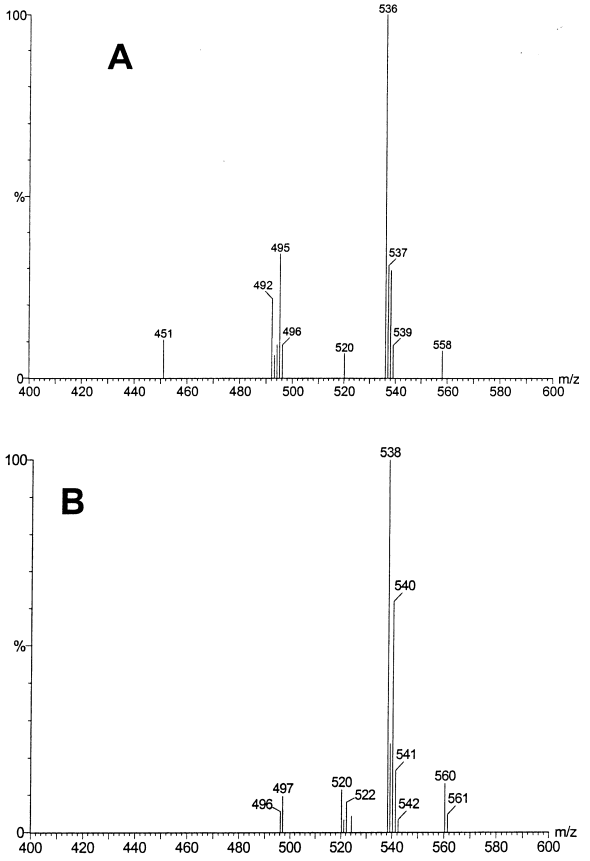


Fig. 1. Negative (A) and positive (B) electrospray ion spectra of lac dye solution.

retention times and peak shapes for individual ions showed that the ion m/z: 492 is derived from laccaic acid A by the loss of 44 (CO_2) through the in-source fragmentation. Similarly, ion m/z: 451 is derived from laccaic acid B by in-source fragmentation (loss of 44). The ion m/z: 558 is possibly a sodium adduct of laccaic acid A ($[M-2H+Na]^-$). This hypothesis is supported by the presence of ion m/z: 560 ($[M+Na]^+$) in the positive ion spectrum, but the chromatographic trace of ion 558 does not fully overlay with that of ion m/z: 536 and the consecutive losses observed in the CID spectrum of ion 558 do not correlate with these observed for ion m/z: 536. The CID spectrum of ion m/z: 558 exhibits the fragmentation pattern of CO_2 and H_2O losses characteristic for laccaic acids. The assignment of ion m/z: 520 in Fig. 2A to xantholaccaic acid A was merely done based on the agreement of the molecular ion mass of xantholaccaic acid A, observed distinct chromatographic peak for ion m/z: 520 (retention time: 14.4 min), and the CID spectrum of ion 520 exhibiting the fragmentation pattern of a laccaic acid. Further structural elucidation work would need to be done to confirm this assignment.

Most of the examined reference substances showed ion formation in both positive and negative electrospray modes. The intensities of pseudomolecular ions obtained for the same solution of a reference substance in the co-infusion experiment were compared in order to determine which mode would be advantageous for the analysis of sample extracts. Generally, the flavanoids showed pseudomolecular ion intensities about four times higher in the positive ion mode than these for negative ions mode. The negative ion mode was advantageous for most of the acids examined, purpurin, alizarin, lawsone, and comparable with positive ion mode for laccaic acids. The electrospray negative ion mode was used for further work with sample extracts, assuming that background contribution would be lower in the negative ion mode and the adduct formation would be limited, and also because of the frequent use of this mode reported in the literature even for flavanoids.

3.2. Analysis of authentic fiber extracts

Fig. 2 presents an example of the identification approach used for carminic acid in the extract of sample of red silk. The initial identification is achieved by the analysis of the UV–Vis (255 nm) and the full scan TIC (total ion chromatogram). The peak at 19.2 min of the UV trace highlights the interesting region of the chromatogram (Fig. 2A), the dominant ion of m/z 491 (Fig. 2B) is identified in the TIC trace in the retention time region of the UV peak. Daughter ion spectrum of the 491 ion is acquired (Fig. 2C) and compared with the spectra of standards. This identifies the unknown peak as the carminic acid. Further conformation was also achieved by comparison of the UV–Vis spectrum of the unknown peak and the retention time when sample was rerun using System II. This identification approach, even though it gives the largest degree of certainty of identification for the techniques used here, is applicable only to samples with relatively large concentrations of the investigated unknown compounds that result in high pseudomolecular ion intensities, yielding a good quality daughter ion spectrum. Using this approach other chemical species, namely, luteolin, apigenin, alizarin, purpurin were identified in other extracts.

Fig. 3 presents another example of identification of unknowns using the daughter ion spectra. The extract of red wool sample shows four major peaks in the UV trace (Fig. 3A1). The first peak at 31.1 min was easily identified as alizarin, based on the daughter ion spectrum of ion m/z: 239, retention time, and UV–Vis spectrum compared with the reference standard. Similarly, the peak at 34.0 min was identified as purpurin. However, the small peak before the purpurin peak on the UV trace and visible in the 239 m/z ion trace (Fig. 3A2) was a true unknown. The daughter ion spectrum for that peak (Fig. 3B) and the retention time did not match any of the examined reference substances. The UV spectrum could not be obtained, as this signal overlapped with the large peak of purpurin, recorded on System II. The identity of the compound eluting at 33.6 min (239 m/z ion trace) was postulated as xanthopurpurin. Novotna et al. [29] reported the presence of xanthopurpurin in historical textiles detected by HPLC–UV. Since xanthopurpurin was not available as a reference and no literature report could be found with the CID spectrum of xanthopurpurin, an attempt was made to assign the ions present in the spectrum in Fig. 3B to the plausible fragmentation pattern of xanthopurpurin. Fig. 4 presents the proposed frag-

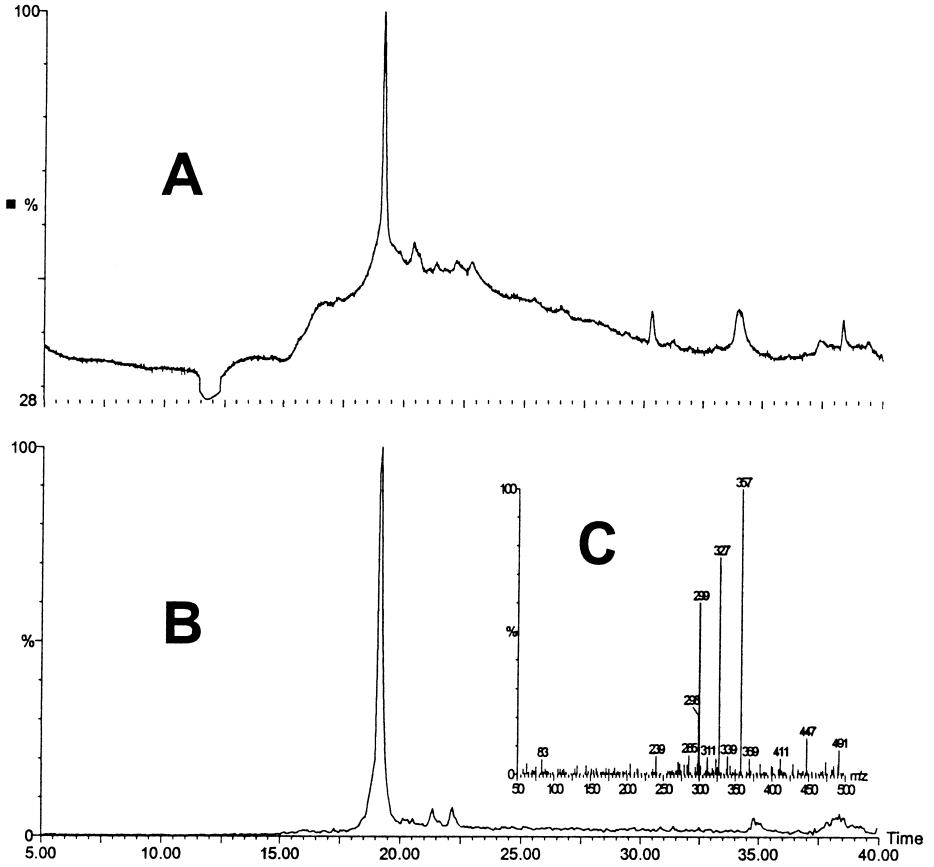


Fig. 2. Identification of carminic acid in sample extract of red silk using System I: (A) UV trace at 255 nm, carminic acid peak at 19.2 min; (B) extracted ion trace for m/z: ion 491 from the full scan ESI chromatogram, carminic acid peak at 19.3 min; (C) daughter ion spectrum of ion m/z: 491.

mentation pattern for xanthopurpurin. The comparison of the alizarin spectrum (Table 1) and the spectrum of unknown postulated as xanthopurpurin (Fig. 3B) indicates that essentially the only difference is seen in the presence of ion 195 m/z in the spectrum of xanthopurpurin. Analysis of the fragmentation mechanisms for flavanoids shows that the presence of two hydroxyl groups in the *meta* position allows the pseudomolecular ion lose CO_2 (44) in the first step of fragmentation. This is observed for xanthopurpurin, but not for alizarin, indirectly supporting the identification of the unknown as xanthopurpurin.

Analysis of MS spectrum for the peak at 35.8 min (Fig. 3A) indicated that this unknown must contain one chlorine. The ratio of the intensity of ions m/z: 273 and 275 match the expected value for a molecule with one chlorine. Similarly, the ratio of ions m/z: 307, 309, 311 for the peak at 36.9 min indicate the presence of two chlorines in the molecule. Additional information was derived from the UV–Vis spectrum. For both peaks the UV spectra closely resembled the spectrum of alizarin. Therefore, it was postulated that the peak at 35.8 min was monochloroalizarin ($[M-H]^- = 273$ m/z) and the peak at 36.9 min was dichloroalizarin (($[M-H]^- = 307$ m/z). Further evidence for the monochloroalizarin was provided through the analysis of the daughter ion spectrum (Fig. 3C) for the peak at 35.8 min. Analysis of the spectrum in Fig. 3C reveals the loss of HCl (36) from the pseudomolecular ion in the first step, followed by four consecutive losses of CO

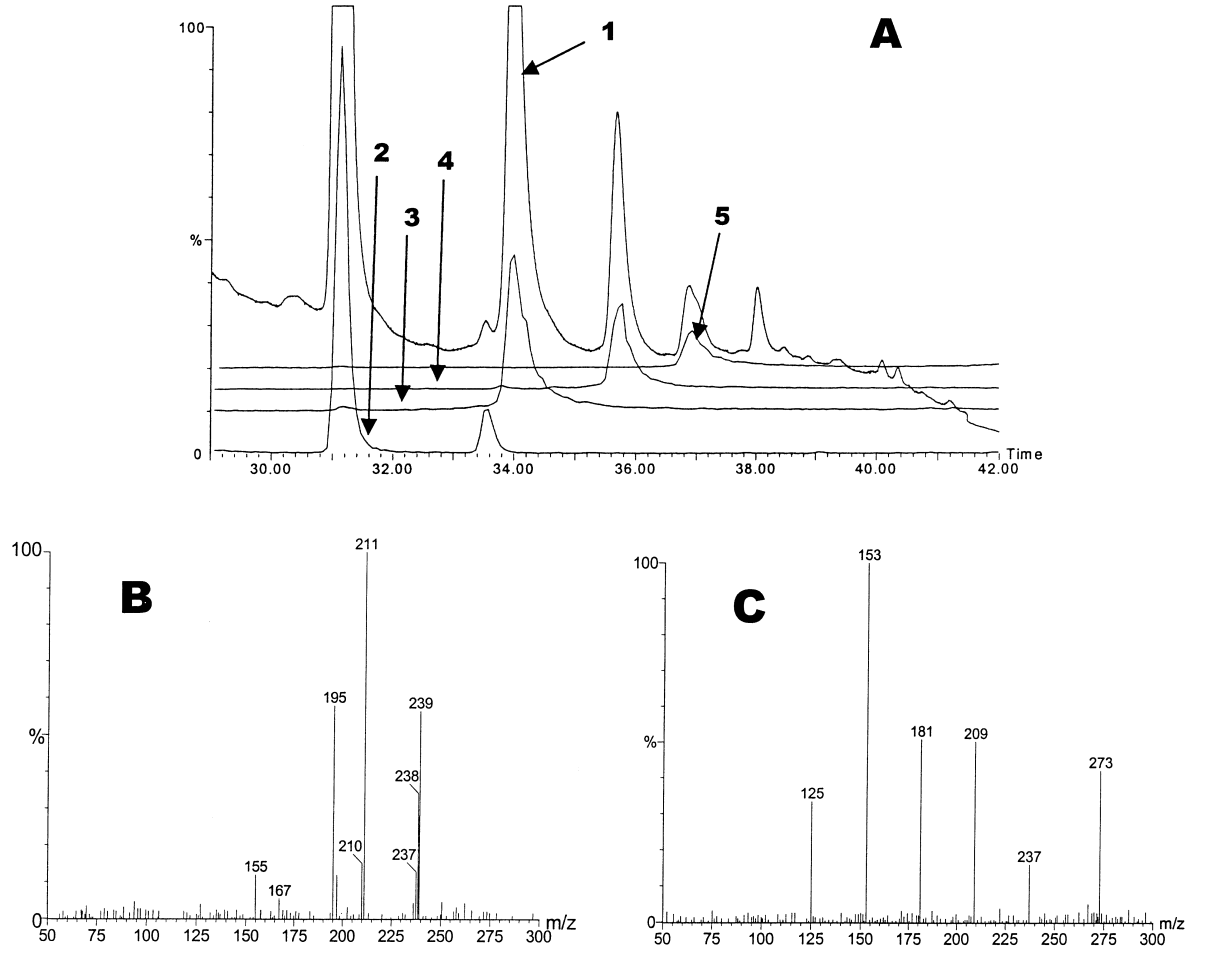


Fig. 3. Identification of unknowns in the extract of sample red wool: (A-1) UV trace at 255 nm; extracted ion trace for ion m/z: 239 (A-2), m/z: 255 (A-3), m/z: 273 (A-4), m/z: 307 (A-5); (B) daughter ion spectrum of m/z: 239 obtained for peak at 33.5 min (C) daughter ion spectrum of m/z: 273 obtained for peak at 35.5 min.

(28), indicating the presence of at least four oxygens in the molecule. Additionally, all other peaks observed in the spectrum are two mass units lower than these observed for alizarin, which would be expected if two hydrogens were removed from alizarin (here HCl from monochloroalizarin). This further supports the identification of this compound as monochloroalizarin. The 307 m/z ion intensity was not sufficient to obtain a good quality daughter ion spectrum.

The identification of dyes in archeological textiles requires the application of analytical techniques that provide best limits of detection (LODs) combined with a large certainty of identification because of the limited sample availability and the importance of obtaining proper identification of chemical species. The dyes in archeological textiles have usually been detected by UV–Vis detection after chromatographic separation and identified by retention time matching and the UV–Vis spectrum. The LODs for selected flavanoids using the UV detection were reported by Andlauer et al. [14] and Crozier et al. [30]. Huck et al. [31] compared LODs for selected flavanoids for UV and MS detection on an ion trap MS. The reported MS LODs were in the μg range and these for UV detection in the ng range. Derksen et al. [32] compared on-line LC with MS and UV–Vis detection for anthraquinones derived from plant extract. The UV–Vis detection was found more sensitive

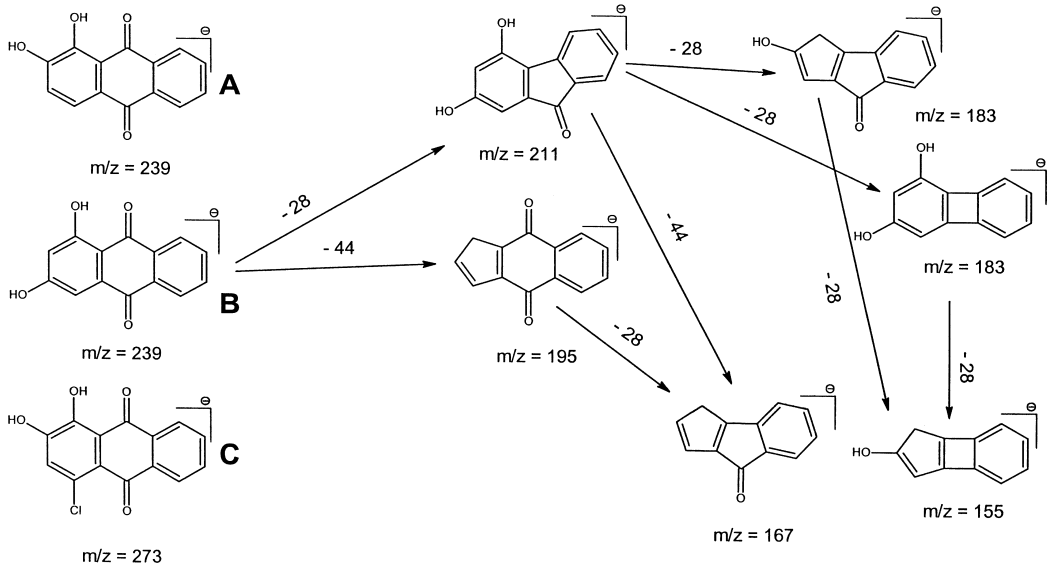


Fig. 4. Proposed fragmentation pattern for xanthopurpurin (B); A, alizarin; C, monochloroalizarin.

Table 2
Summary of the signal-to-noise ratios obtained for the UV–Vis and MS–MS detection of selected compounds detected in the extracts of Coptic fiber samples

Sample	Luteolin (UV[a] 348 nm; MRM[b] 285→ 133 *m/z*)	Apigenin (UV 337 nm; MRM 269→ 117 *m/z*)	Rhamnetin (UV 368 nm; MRM 315→ 121 *m/z*)	Alizarin (UV 427 nm; MRM 239→ 210 *m/z*)	Purpurin (UV 480 nm; MRM 255→ 129 *m/z*)	Indirubin (UV 290 nm; MRM 263→ 219 *m/z*)
Brown wool AD 4th	UV <3 MRM 15	UV ND[c] MRM 8.7	UV ND MRM 9.7	UV 268 MRM 146	UV 400 MRM 930	UV ND MRM 6.0
Red wool AD 7th–9th	UV 100 MRM 77	UV ND MRM 24	UV ND MRM 7.5	UV 140 MRM 155	UV 170 MRM 460	UV ND MRM ND
Red silk Unknown date	UV 42 MRM 29	UV ND MRM 14	UV ND MRM ND	UV 10 MRM 20	UV 89 MRM 82	UV ND MRM ND
Red wool Unknown date	UV <3 MRM 42	UV 9.0 MRM 8.0	UV ND MRM ND	UV 600 MRM 500	UV 97 MRM 92	UV ND MRM ND
Green wool AD 7th 9th	UV 18 MRM 114	UV ND MRM 45	UV 5 MRM 17	UV 69 MRM 59	UV 38 MRM 26	UV 14 MRM 11
Brown wool AD 3rd–4th	UV ND MRM 8.3	UV ND MRM ND	UV ND MRM ND	UV 650 MRM 510	UV 240 MRM 150	UV 9.7 MRM 24
Dark yellow wool AD 6th	UV 210 MRM 250	UV 30 MRM 190	UV ND MRM 25	UV 330 MRM 240	UV 72 MRM 120	UV ND MRM ND
Green wool AD 6th	UV 290 MRM 470	UV 36 MRM 155	UV ND MRM 7.0	UV 130 MRM 115	UV 10 MRM 18	UV 9.0 MRM 29

[a] Indicates the wavelength used for signal-to-noise ratio calculation.
[b] Indicates the masses of ions used for the multiple reaction monitoring (MRM) transition.
[c] ND, not detected.

than MS detection for the investigated anthraquinones.

The work presented here involves simultaneous analysis of the extracts using UV–Vis and MS detection utilizing different scanning modes of a triple quadrupole mass spectrometer. Comparison of the detection modes is done for the same extract within the same chromatographic run. However, this work was of a qualitative nature and the tuning parameters of the MS were not fully optimized for individual analytes to obtain maximum sensitivity. In order to further investigate the detection capability of the UV–Vis and MS detections, the S/N ratios for selected analytes were calculated and are presented in Table 2. The six selected analytes span the range of compounds belonging to flavanoids, anthraquinones, and indigoids. The S/N ratios were calculated using the MASSLYNX algorithm with the RMS noise evaluation for a nonsmoothed signal. The S/N ratios obtained for luteolin, apigenin, and rhamnetin were higher for the MS detection for the majority of examined sample extracts. Purpurin, alizarin, and indirubin showed largely equivalent S/N ratios for UV–Vis and mass spectrometric detection. Fig. 5 illustrates the data presented in Table 2, where both the UV–Vis and the MS–MS (MRM mode) detection of luteolin, apigenin, and rhamnetin obtained for the extract of green wool, using System II, are presented. The MRM traces were smoothed for the presentation. It is evident that the S/N ratios for luteolin, apigenin, and rhamnetin are superior to these obtained for the UV detection.

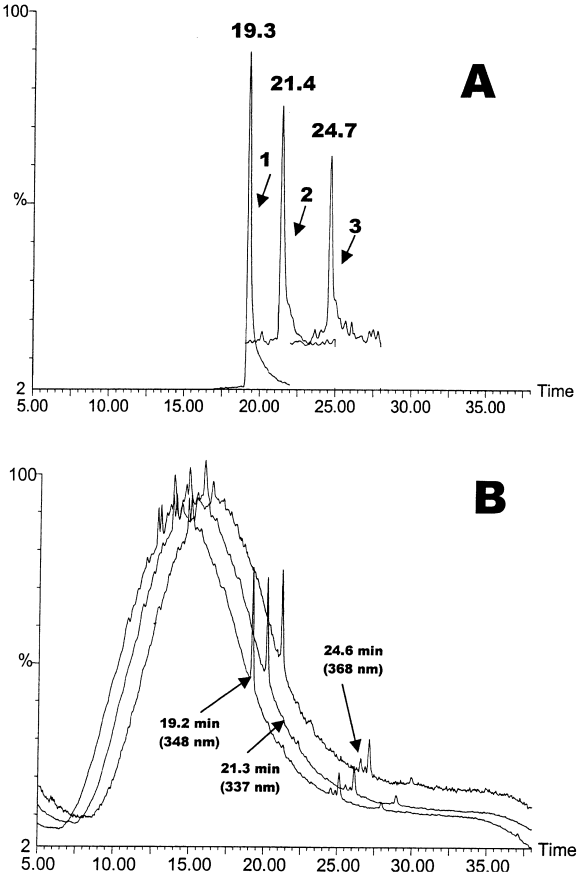


Fig. 5. Comparison of detection of luteolin (19.3 min), apigenin (21.4 min), and rhamne-tin (24.7 min) in the extract of green wool: MRM trace for monitored transition: 285 to 133 m/z (A-1); 269 to 117 m/z (A-2); 315 to 121 m/z (A-3); (B) UV traces at 348 nm (luteolin), 337 nm (apigenin), 368 nm (rhamnetin). The UV traces were shifted by 1 min each for clear presentation.

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References

[1] J. Wouters, Studies Conservation 30 (1985) 119.
[2] J. Wouters, A. Verhecken, Studies Conservation 34 (1989) 189.
[3] W. Nowik, Analusis 24 (1996) M37.
[4] C.H. Fischer, M. Bischof, J.G. Rabe, J. Liq. Chromatogr. 13 (1990) 319.
[5] A. Hayashi, M. Saito, Bunkazai Hozon Shufuku Gakkaishi 45 (2001) 27.
[6] Z.C. Koren, J. Soc. Dyers Colourists 110 (1994) 273.
[7] J. Wouters, Dyes History Archeol. 13 (1994) 38.
[8] A. Quye, J. Wouters, Dyes History Archeol. 10 (1992) 48.
[9] S.R. Yamaoka, N. Shibayama, T. Yamada, M. Sato, Shitsuryo Bunseki 37 (1989) 249.
[10] E.S.B. Ferreira, A. Quye, H. McNab, A.N. Hulme, J. Wouters, J.J. Boon, Dyes History Archaeol. 16/17 (2001) 179.

[11] E.S.B. Ferreira, A. Quye, H. McNab, A.N. Hulme, Dyes History Archaeol. 18 (2002) 63.
[12] R.H. Michel, J. Lazar, P.E. McGovern, J. Soc. Dyers Colourists 108 (1992) 145.
[13] W. Nowik, Encyclopedia of Separation Science, Academic Press, London, 2000, p. 2602.
[14] W. Andlauer, M.J. Martena, P. Furst, J. Chromatogr. A 849 (1999) 341.
[15] S.M. Halpine, Studies Conservation 41 (1996) 76.
[16] J. Wouters, A. Verhecken, J. Soc. Dyers Colourists 107 (1991) 266.
[17] Z.C. Koren, Dyes History Archeol. 11 (1993) 25.
[18] K. Urbaniak-Walczak, Koptische Stoffe aus der Sammlung des Nationalmuseums in Warschau: Gaschichte der Sammlung, in: S. Emmel, M. Krause, S.G. Richter, S. Schaten (Eds.), Ägypten und Nubien in spätantiker und christlicher Zeit, Reichert, Wiesbaden, 1999, pp. 401–410.
[19] U. Justesen, P. Knuthsen, T. Leth, J. Chromatogr. A 799 (1998) 101.
[20] M. Toyoda, K. Tanaka, K. Hoshino, H. Akiyama, A. Tanimura, Y. Saito, J. Agric. Food Chem. 45 (1997) 2561.
[21] S.E. Nielsen, R. Freese, C. Cornett, L.O. Dragsted, Anal. Chem. 72 (2000) 1503.
[22] R.J. Hughes, T.R. Croley, C.D. Metcalfe, R.E. March, Int. J. Mass Spectrom. 210/211 (2001) 371.
[23] U. Justesen, J. Chromatogr. A 902 (2000) 369.
[24] Y.L. Ma, Q.M. Li, H. Van den Heuvel, M. Claeys, Rapid Commun. Mass Spectrom. 11 (1997) 1357.
[25] Y.L. Ma, H. Van den Heuvel, M. Claeys, Rapid Commun. Mass Spectrom. 13 (1999) 1932.
[26] N. Fabre, I. Rustan, E. de Hoffmann, J. Quetin-Leclercq, J. Am. Soc. Mass Spectrom. 12 (2001) 707.
[27] H. Oka, Y. Ito, S. Yamada, T. Kagami, J. Hayakawa, K. Harada, E. Atsumi, M. Suzuki, M. Suzuki, H. Odani, S. Akahori, K. Maeda, H. Nakazawa, Y. Ito, J. Chromatogr. A 813 (1998) 71.
[28] R. White, J. Kirby, in: J. Kirby (Ed.), Dyes in History and Archeology, Vol. 16/17, The National Gallery, London, 1999, p. 167.
[29] P. Novotna, V. Pacakova, Z. Bosakova, K. Stulik, J. Chromatogr. A 863 (1999) 235.
[30] A. Crozier, E. Jensen, M.E.J. Lean, M.S. McDonald, J. Chromatogr. A 761 (1997) 315.
[31] C.W. Huck, M.R. Buchmeiser, G.K. Bonn, J. Chromatogr. A 943 (2001) 33.
[32] G.C.H. Derksen, H.A.G. Niederlander, T.A. van Beek, J. Chromatogr. A 978 (2002) 119.