Journal of Chromatography A, 1218 (2011) 5837–5847

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Historical and archaeological textiles: An insight on degradation products of wool and silk yarns☆

Ilaria Degano[a,*], Magdalena Biesaga[b], Maria Perla Colombini[a], Marek Trojanowicz[b]

[a] Università di Pisa, Dipartimento di Chimica e Chimica Industriale, via Risorgimento, 35, I-56126 Pisa, Italy
[b] University of Warsaw, Department of Chemistry, Pasteura 1, 02-093 Warsaw Poland

ARTICLE INFO

Article history:
Received 14 January 2011
Received in revised form 17 June 2011
Accepted 27 June 2011
Available online 3 July 2011

Keywords:
Natural dyes
HPLC–ESI-MS
HPLC–APCI-MS
Textile ageing

ABSTRACT

The characterisation of micro-samples from works of art and archaeological residues is a particularly complex task, due to the fact that only a relatively low amount of material is available for sampling, and compounds both derived from the target analytes and the matrix can be simultaneously present. Thus, sensitive, selective and reliable analytical procedures need to be developed. This paper presents the optimisation of an instrumental procedure based on liquid chromatography with mass spectrometric detection, which allows for determining selected analytes (anthraquinones, tannins, flavonoids), along with their known degradation products: phenolic acids. The instrumental parameters were optimised in terms of selecting the best ionisation source (APCI and ESI were compared), choosing the compound-dependant MS parameters and enhancing selectivity and sensitivity (SIM and MRM analyses were compared). The proposed procedure proved to be sensitive and selective, with limits of detection (0.4–20 ng/mL). The analytical procedure was validated by characterising reference materials, i.e. dyed and undyed woollen and silk yarns, both freshly prepared and artificially aged. Particularly, the study focused on the determination of 4-hydroxybenzoic acid, and on the correlation between its relative amounts with respect to ageing time. The optimised procedure was also applied to historical samples and proved fundamental in unravelling the complex composition of black dyed yarns collected from a medieval tapestry. The much degraded yarns were dyed with a tannin based dye, extracted from gall-nuts, alder bark or sumac; the less degraded ones were coloured by superimposing colours with cochineal, madder, weld and indigo dye baths, and eventually by adding gallo-tannins as well.

1. Introduction

Since pre-historical times, mankind has searched for materials for colouring skin, painting, and for dyeing [1,2]. Nature itself offers an amazingly wide range of possible sources of colorants, which can either be inorganic and organic. Their identification is an interesting topic, due to the importance of colour in all the cultures. For dyeing purposes, organic dyes of vegetal and animal origin were employed through human history. Their characterisation is a challenging task for chemists, due to three main problems: the wide range of possible dye source and the vast number of chemical classes they belong to; the small amount of sample available for analysis, and the low amount of coloured compounds (chromophore-containing molecules) in them; the low stability of chromophore-containing molecules themselves and the complexity of their degradation processes (interconnected to the matrix degradation processes) [3].

The identification of dyeing sources is usually achieved by comparing analytical data obtained for unknown samples to the ones obtained for known dyeing sources. Thus, it is of paramount importance to study reference materials, possibly subjected to artificial ageing. Fading of coloured fibres and oxidation of the textile matrix heavily affects the appearance and mechanical properties of textile artefacts; thus, the comprehension of photo-oxidation chemical pathways and fading mechanisms is needed to develop proper preventive conservation strategies.

In most cases, accelerated ageing procedures are performed in order to assess fading rates and identify the factors concurring in fastness [4–8]. On the contrary, very few articles deal with the identification of degradation products in historical samples or even in accelerated aged specimens. Relevant studies were published on the morphology of degraded archaeological textiles, characterised by means of scanning electron microscopy (SEM) equipped with an energy dispersive X-ray spectrometer (EDS) [9].

With regard to the study of oxidation pathways, the most studied chromophore-containing molecules are flavonoids [10–12,28,36]. Hydroxybenzoic acids were detected in the extracts

☆ Presented at LCMS 2008: 25th LC/MS Montreux Symposium: Historical Perspectives and Future Outlook, Montreux, Switzerland, 12–14 November 2008.

* Corresponding author. Tel.: +39 050 2219258; fax: +39 050 2219260.
E-mail addresses: ilariad@dcci.unipi.it (I. Degano), mbiesaga@chem.uw.edu.pl (M. Biesaga), perla@dcci.unipi.it (M.P. Colombini), trojan@chem.uw.edu.pl (M. Trojanowicz).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.06.095

from aged specimens. Their presence may be due to the photo-oxidation of the double bond C2–C3 of the flavonoids, leading to the formation of a depside (depsides are formed by condensation of two or more hydroxybenzoic acids whereby the carboxyl group of one molecule is esterified with a phenolic hydroxyl group of a second molecule), which in turn gives low-molecular mass products, such as dihydroxybenzoic acids and trihydroxybenzoic acids. Possible degradation pathways (one involving the mordant metallic ion and the other the light as a catalyst) are suggested in [13] and some of the hypothesised degradation products were also identified in historical and archaeological samples [14,15]. Madder degradation was also investigated, due to its wide occurrence in works of art. Ahn and Obendorf [16] studied alizarin in aqueous solution and identified possible degradation products such as phtalic acid and phtalic anhydride, and benzoic acid, while Clementi et al. [17] did not identify any degradation product, after accelerated ageing of wool yarns dyed with madder.

With regard to the textile matrix, in accordance with the literature, ageing effects in wool and silk are mainly due to alterations in the protein fraction. Particularly, wool interacts with UV radiation through histidine, tryptophan, tyrosine, methionine, cystine and cysteine residues, and silk through tryptophan, tyrosine and phenylalanine. Amino acid analysis of aged silk yarns [18] showed that tyrosine is the least stable amino acid followed by arginine, tryptophan, lysine, aspartic and glutammic acid and serine. Photochemical studies on wool and silk mainly focus on the reaction of cysteine and cystine to give cysteic acid [19], and on the reactions of aromatic amino acids, i.e. tryptophan and tyrosine [20,21]. The latter may react to yield phenoxyl radicals and then products of oxidative coupling, e.g. di-tyrosine, etc. The excited singlet state of tyrosine may also be quenched by a neighbouring cystyl residue, resulting in a cleavage of the disulphide bond, whereas tryptophan, if irradiated at short wavelengths, may undergo electron ejection leading to the formation of a 3-indolyl radical [22]. It is reported that irradiation of wool generates singlet oxygen [22] and hydroperoxides. As a consequence, phenoxyl radicals might be generated from phenols, leading to the hydroxylation of aromatic compounds such as tyrosine, phenylalanine and tryptophan: α-ketocarboxilic acids might be formed from tyrosine and quinones and related species from tryptophan. These products of photo-oxidation are considered to be responsible for yellowing [23].

The study of such complex ageing processes and the characterisation of dyeing mixtures is only possible thanks to the increasing availability of highly specific and sensitive analytical procedures, which were applied to the research on organic dyes in cultural heritage related artefacts [24]. In particular, the application of micro destructive methods entailing a solvent extraction from the matrix (i.e., lake substrate or yarns), followed by separation techniques, was the choice of election for their characterisation in most of the cases. The target chromophore-containing molecules are polar, water-soluble molecules; thus, the most widely used technique is reverse phase liquid chromatography HPLC with diode array detectors [25,26] often coupled with MS detectors [27–38]. Fluorescence spectrophotometers were also used as sensitive and selective detectors [39–41].

Besides its high selectivity, MS detection is extremely useful for its sensitivity, which makes it the perfect choice for the analysis of organic dyes in micro-samples.

Nonetheless, the polarity of the target analytes ranges from high hydrophilicity (mordant and direct dyes) to relatively high hydrophobicity (indigoid dyes), and thus the choice of the proper ionisation source is an extremely critical step. As a consequence, most of the published articles specifically deal with only one or two classes of dyes by ESI or APCI ionisation source.

In principle, in order to set up an analytical procedure, the best ionisation source has to be chosen, and instrumental parameters both dependant on the nature of the target compounds and on the chromatographic separation have to be optimised. This will result in better ionisation yields and best peak shapes, and ultimately for lower LODs [27–38]. Limits of detection for hydroxybenzoic acids, flavonoids and anthraquinones reported in the literature may be summarised as:

- For ESI-MS and ion trap analysers, positive mode and SIM detection: 10–150 ng/mL [31].
- For ESI-MS and ion trap analysers, for negative mode and SIM detection: 30–90 ng/mL [42].
- For ESI-MS and quadrupole analyser, in negative mode: 5 ng/mL [43].
- For APCI-MS and quadrupole analyser, in negative mode: 20 ng/mL [43].
- for ESI-MS, negative mode and SIM detection: 0.5–12 ng/mL [28].

This works comprises a detailed study of selected chromophore-containing molecules belonging to different chemical classes (indigoids, flavonoids, anthraquinones and tannins) using high performance liquid chromatography/mass spectrometry with both APCI and ESI ion sources. The purpose of this study was to fill in the gaps in the literature by comparing the two ion sources, different ionisation modes (positive and negative) and acquisition methods. The final target is to propose a protocol optimised in terms of selectivity and sensitivity. At present, systematic comparisons of APCI and ESI ionisation sources and of SIM and MRM acquisition methods have never been described. The only example of the optimization of detection modes is given by Surowiec et al. [28], who used an ESI source and compared different acquisition modes, namely, scanning, SIM, and multiple reaction monitoring (MRM) in both positive and negative ion modes, for the detection of anthraquinones, flavonoids and hydroxybenzoic acids.

Moreover, the proposed analytical procedure has been validated by analysing reference dyed and undyed woollen and silk yarns, also subjected to accelerated ageing. In particular, undyed mordanted wool and silk yarns were studied in order to clarify the nature of the degradation products originating from the matrix.

The application of the proposed HPLC–MS procedure proved fundamental in unravelling the complex composition of black dyed yarns collected from a 15th century tapestry, The final attack on Jerusalem, Tournai manufacture (Hainaut, Belgium).

2. Materials and methods

2.1. Reagents

All the chemicals were of HPLC gradient grade from Merck (Darmstadt, Germany). Ultra pure water from Milli-Q system (Millipore, Bedford, MA, USA) with conductivity of 18 MΩ/cm was used in all experiments.

Formic acid (>98%) was obtained from Riedel-de Haen (Seelze, Germany) and hydrochloric acid (25%) was from Merck (Darmstadt, Germany).

Standard solutions of chromophore-containing molecules for analysis in infusion mode were prepared in methanol from alizarin (85%), apigenin (approx. 95%), carminic acid, and morin from Sigma–Aldrich (Steinheim, Germany); from xanthopurpurin and anthragallol from H. Schweppe (Frankenthal, Germany); from emodin and purpurin (techn., F90%) from Fluka (Buchs, Switzerland); from luteolin and rhamnetin from Roth (Karlsruhe, Germany); from gallic acid, kaempferol, genistein, naringenin, naringin, quercetin and rutin from Sigma (Steinheim, Germany); from 3,4-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid (from Acros Organics, Geel, Belgium). Stock

solutions of indigoids were prepared in dimethyl sulfoxide (DMSO) from indigotin, indirubin, 6,6′-dibromo indigotin from Merck (Darmstadt, Germany).

The solutions used for calibration were 50, 130, 250, 500, 1000, 2500 and 5000 ng/mL in methanol.

All solutions were filtered through 0.45 μm membranes (Millipore). All reagents and chemicals were used without any further purification.

2.2. Instrumentation

2.2.1. HPLC with double wavelength UV–vis detector and MS detector

An HPLC-UV–vis Shimadzu (Japan) with a two wavelengths UV detector coupled to MS 3200 Q Trap (Applied Biosystems, USA) was used. The separations were carried at 30 °C on Zorbax RX-C8, 2.1 mm × 150 mm, 5 μm particle C8 column (Agilent, CA, USA). Injection volume was 20 μL. Mobile phase consisted in (A) 0.3% v/v formic acid in water (pH = 2.4) and (B) acetonitrile. The elution gradient was: 0–2 min, 5% B; 2–30 min, linear gradient to 60% B; 30–35 min, linear gradient to 100% B; hold for 10 minutes. Flow rate was 0.2 mL/min in order to interface MS detection with chromatographic system. Working wavelengths were 254 nm and 275 nm. Data were processed with Analyst 4.2 software.

MS 3200 Q Trap allows for using both ESI and APCI ion sources, and for different scanning modes: MS^2, MS^n ($n > 2$ if the last quadrupole is used as an ion trap, LIT) and Multiple Reaction Monitoring (MRM).

The instrumental conditions differ for ESI and APCI. For ESI, the source temperature was set at 400 °C, the ion spray voltage at +5.5 kV for positive and −4.5 kV for negative ionisation mode, the curtain gas pressure was 0.07 MPa and the nebuliser gas pressure 0.3 MPa. For APCI, the source temperature was set at 400 °C, the nebulizer current at +3.0 μA for positive and −3.0 μA for negative ionisation mode, the curtain gas pressure, the nebuliser gas pressure and the auxiliary gas pressure were all set at 0.3 MPa. Nitrogen was used as curtain and auxiliary gas and the flow was set at 10 mL/min for ESI source and 45 mL/min for APCI source. The optimal detection conditions for MRM were determined in infusion mode. Standard solutions were infused into the source via a 50 μm i.d. peek capillary using an Harward Apparatus pump at 10 μL/min. Continuous mass spectra were obtained by scanning in the range from 50 to 650 *m*/*z*.

2.2.2. Ageing chamber

Artificially accelerated ageing was performed in a "Solar Box" (Solar Box 1500e RH, Erichsen, Italy). Accelerated ageing was performed with an outdoor filter (280 nm). The ageing conditions were as follows: 35 ± 5 °C, relative humidity (RH) 50%, radiance: 500 MJ/m^2.

2.3. Reference materials

2.3.1. Reference raw materials and dyed specimens

Persian berries (*Rhamnus catharticus*) were purchased from Kremer-Pigmente (Germany). Two types of raw wool were employed: one is an Italian, commercial wool, 4/10 "pura lana", cream colour (Campolmi, Italy); the second one was purchased directly from a craftsman in Bielsk Podlaski (Bielsk, Poland). Reference raw silk yarns were provided from Opificio delle Pietre Dure ("seta schappe"). Alum was purchased from "Zecchi Colori belle arti restauro" (Florence, Italy).

Reference yarns were mordanted and/or dyed in laboratory. Both wool species were mordanted with alum; Polish wool was also dyed with Persian berries. The mordanting and dyeing procedure is based on a previously published one [11,43,44] and is described as follows:

- *Preliminary scouring*: 15 g of raw wool were immersed in 160 mL deionised water containing 0.75 g of Triton X100 (octyl phenol ethoxylate, a nonionic surfactant). The scouring bath was heated up to 40 °C and left standing for 30 min. The wool was rinsed with deionised water.
- *Mordanting*: 3.6 g of alum and 0.9 g of cream of tartar were dissolved in 750 mL of water. The scoured woollen yarns were immersed in the solution and heated up to 90 °C for 30 min. The yarns were rinsed and allowed to dry overnight in the dark.
- Dyeing: 7 g of the raw colorant was soaked in 50 mL of deionised water overnight; the solution was filtered and the procedure was repeated twice in order to prepare the dye bath. The mordanted wool (1.2 g of dried yarn) was immersed in the dye bath that was then heated up to 90 °C for 30 min. The dye bath was then allowed to cool for 30 min and the dyed yarns were rinsed with deionised water, and then stored in a dark place to dry overnight.

Subsequently, the yarns were aged in the Solar Box. Three sets of accelerated ageing experiments were performed. The wool "lana Campolmi", mordanted with alum, was sampled after 48, 168, 336 and 504 h of accelerated ageing. The Polish wool, both unmordanted and alum-mordanted, and the "seta Schappe" were sampled after 188, 216, 360 and 1130 h of accelerated ageing. The Polish wool, alum-mordanted and dyed with Persian berries, was sampled after 24, 197, 385 and 1000 h of accelerated ageing.

2.3.2. Historical samples

A 15th century tapestry, *The final attack on Jerusalem* (dated 1480), Tournai manufacture (Hainaut, Belgium), was studied in the frame of a conservation project in progress at the Opificio delle Pietre Dure (Florence, Italy). Samples are listed in Table 1. Violet, red, orange, blue, green and beige areas were sampled in order to characterise the dyeing palette. With regard to black areas, two different kinds of materials were classified by the restorers: very degraded and darker yarns could be distinguished from less degraded and slightly lighter ones. From a careful study of weaving and of the quality of threads, restores hypothesised that the lighter yarns were original, while the darker ones were added during an 18th century restoration. Very degraded samples belonging to the two classes of blacks were analysed to identify the colouring materials in order to validate the hypothesis of the restorers.

Table 1
Description of samples collected by OPD restorers from *The final attack on Jerusalem tapestry* (1480, Tournai manufacture).

Sample *n*	Colour	Sample weight (mg)
IV m′	Blue	1.3
IV s	Red	2.2
III H′	Dark brown	0.8
I 28	Black	1.8
I 29	Black	2.9
III 19	Dark brown	1.4
III 33	Black	3.9
III 40	Black	1.9
I Q*	Black	1.3
III S*	Brown	0.8

2.4. Sample treatment

The samples collected from reference specimens (1.5–4 mg) and from the historical tapestry (see Table 1) were treated with 300 μL HCl/MeOH/H_2O (2:1:1) in open glass vials for 10 min at boiling point; the extract was dried under nitrogen gas. The samples were reconstituted in 50 μL DMSO and purified on nylon filters

Table 2
Mass fragments of target analytes acquired with ESI and APCI sources.

Compound	MW	Ion source	Ion mode	MS/MS ions *m*/*z* (relative abundance, %)
4-Hydroxy benzoic acid	138	ESI	+	95 (100), 121 (62), 93 (20), 97 (4)
			–	93 (100), 119 (8), 96 (3)
		APCI	+	79 (100), 81 (7)
			–	93 (100)
2,4-Dihydroxy benzoic acid	154	ESI	+	81 (100), 137 (69), 95 (28), 119 (20)
			–	109 (100), 135 (0.5)
		APCI	+	95 (100), 123 (62), 137 (29), 109 (15)
			–	109 (100), 135 (30), 91 (17), 108 (3)
3,4-Dihydroxy benzoic acid	154	ESI	+	93 (100), 111 (51), 137 (27), 81 (17)
			–	109 (100), 108 (25), 91 (5)
		APCI	+	123 (100), 95 (76), 93 (11), 111 (9)
			–	109 (100), 108 (20), 107 (5), 91 (4)
Gallic acid	170	ESI	+	127 (100), 153 (89), 109 (83), 81 (75)
			–	125 (100), 79 (8), 81 (6), 97 (5)
		APCI	–	125 (100), 97 (11), 79 (10), 81 (6.5)
Xanthopurpurin	240	ESI	+	115 (100), 199 (91), 143 (52), 139 (45)
			–	211 (100), 195 (89), 210 (29), 167 (20)
		APCI	–	211 (100), 195 (93), 210 (34), 167 (23)
Alizarin	240	ESI	+	85 (100), 163 (72), 103 (5)
			–	210 (100), 211 (84), 167 (39), 101 (26), 127 (21)
		APCI	–	210 (100), 211 (72), 167 (34), 155 (21), 101 (20), 127 (16)
Purpurin	256	ESI	+	229 (100), 187 (86), 127 (79), 103 (64)
			–	227 (100), 129 (67), 101 (59), 171 (28), 183 (20)
		APCI	+	229 (100), 127 (99), 187 (90), 159 (47)
			–	227 (100), 129 (52), 101 (52), 171 (24), 183 (15)
Anthragallol	256	ESI	+	127 (100), 155 (67), 183 (67), 211 (59)
			–	125 (100), 153 (73), 171 (45), 143 (40), 227 (35)
		APCI	–	125 (100), 153 (49), 143 (35), 171 (33)
Indigotin	262	APCI	–	156 (100), 217 (67), 261 (30), 260 (24)
Indirubin	262	ESI	–	157 (100), 217 (15), 156 (13)
		APCI	–	157 (100), 217 (13), 156 (41)
Emodin	270	ESI	+	115 (100), 197 (47), 141 (39), 169 (38)
			–	225 (100), 241 (32), 182 (24), 197 (22)
		APCI	–	225 (100), 241 (33), 182 (25), 181 (25)
Apigenin	270	ESI	+	153 (100), 119 (50), 91 (48), 121 (19)
			–	117 (100), 151 (36), 149 (26), 107 (23)
		APCI	+	153 (100), 91 (49), 119 (46), 121 (17)
			–	117 (100), 151 (33), 107 (23), 149 (22)
Genistein	270	ESI	+	91 (100), 153 (84), 215 (54), 115 (39), 197 (26), 141 (23), 119 (16), 121 (10)
			–	133 (100), 132 (44), 135 (38), 159 (32), 157 (15), 224 (15), 225 (12), 181 (10)
		APCI	+	91 (100), 153 (70), 215 (58), 115 (38), 243 (32), 149 (25), 141 (23), 253 (18)
			–	133 (100), 135 (48), 107 (45), 132 (41), 181 (35), 183 (28), 91 (28), 89 (10)
Naringenin	272	ESI	+	153 (100), 147 (48), 91 (26), 119 (23)
			–	151 (100), 119 (97), 107 (39), 83 (18)
		APCI	+	153 (100), 147 (54), 91 (27), 119 (27)
			–	119 (100), 151 (58), 107 (33), 83 (18)
Kaempferol	286	ESI	+	153 (100), 121 (59), 165 (35), 213 (22)
			–	93 (100), 117 (84), 151 (64), 185 (54)
		APCI	+	153 (100), 121 (59), 165 (32), 93 (26)
			–	151 (100), 93 (45), 107 (38), 108 (35)
Luteolin	286	ESI	+	115 (100), 89 (83), 87 (72), 139 (35)
			–	151 (100), 133 (57)
		APCI	+	153 (100), 89 (63), 135 (50), 117 (25)
			–	133 (100), 132 (34), 151 (24), 107 (14)
Morin	302	ESI	+	153 (100), 137 (65), 229 (52), 149 (44)
			–	151 (100), 125 (72), 149 (52), 107 (40)
		APCI	+	153 (100), 137 (59), 229 (55), 149 (45)
			–	151 (100), 125 (69), 83 (43), 149 (35)
Quercetin	302	ESI	+	153 (100), 229 (67), 137 (61), 165 (39)
			–	151 (100), 179 (41), 107 (32), 121 (30)
		APCI	+	153 (100), 229 (60), 137 (59), 165 (42)
			–	151 (100), 179 (31), 121 (25), 107 (21)
Rhamnetin	316	ESI	+	123 (100), 274 (51), 167 (49), 243 (36)
			–	165 (100), 121 (47), 97 (19), 300 (17)
		APCI	+	123 (100), 167 (56), 274 (49), 243 (38)
			–	165 (100), 121 (45), 97 (16), 300 (15)
6,6′-Dibromoindigotin	420	ESI	+	403 (100), 389 (78), 147 (43), 361 (42)
		APCI	–	79 (100), 81 (93), 339 (14), 337 (9)
Carminic acid	492	ESI	–	357 (100), 327 (97), 299 (81), 447 (80)
		APCI	+	355 (100), 373 (74), 325 (23), 379 (18)
			–	357 (100), 327 (77), 299 (75), 447 (54)
Naringin	580	ESI	–	151 (100), 271 (74), 119 (49), 107 (29)
		APCI	+	273 (100), 153 (55), 85 (23), 147 (51)
			–	151 (100), 271 (67), 117 (49), 107 (33)
Rutin	610	ESI	–	300 (100), 271 (48), 301 (37), 255 (23)
		APCI	+	303 (100), 465 (16), 85 (15), 153 (5)
			–	300 (100), 271 (45), 255 (24), 243 (17)

Table 3
SIM and MRM acquisition parameters for target analytes, negative mode and ESI source (DP, cone voltage/declustering potential; CE, collision energy).

Compound	SIM acquisition m/z value	Acquired MRMs	DP (V)	CE (V)
4-Hydroxybenzoic acid	137	137 → 93	−25	−18
		137 → 119	−25	−8
2,4-Dihydroxybenzoic acid	153	153 → 109	−25	−18
3,4-Dihydroxybenzoic acid	153	153 → 109	−35	−20
Gallic acid	169	169 → 125	−45	−20
		169 → 97	−45	−26
Xanthopurpurin	239	239 → 211	−55	−34
		239 → 195	−55	−34
Alizarin	239	239 → 210	−38	−55
		239 → 167	−36	−55
Purpurin	255	255 → 227	−50	−34
		255 → 183	−50	−36
Anthragallol	255	255 → 153	−55	−40
		255 → 227	−55	−34
Indirubin	261	261 → 157	−65	−40
		261 → 217	−65	−32
Emodin	269	269 → 225	−60	−34
		269 → 241	−60	−36
Apigenin	269	269 → 117	−50	−46
		269 → 151	−50	−34
Genistein	269	269 → 133	−60	−42
		269 → 181	−60	−26
Naringenin	271	271 → 119	−50	−34
		271 → 151	−50	−26
Kaempferol	285	285 → 151	−70	−28
		285 → 185	−70	−36
Luteolin	285	285 → 151	−80	−30
		285 → 133	−80	−38
Morin	301	301 → 151	−45	−28
		301 → 125	−45	−28
Quercetin	301	301 → 151	−70	−30
		301 → 179	−70	−26
Rhamnetin	315	315 → 165	−70	−30
		315 → 300	−70	−28
Carminic acid	491	491 → 357	−55	−34
		491 → 447	−55	−24
Naringin	579	579 → 271	−70	−46
		579 → 151	−80	−54
Rutin	609	609 → 300	−80	−48
		609 → 271	−80	−72

(Millipore); 10 μL were injected in the chromatographic system (procedure adapted from [17,28,45]).

3. Results and discussion

3.1. Optimisation of MS detection and acquisition parameters

The optimisation of MS acquisition was achieved through the assessment of the best compound dependant parameters: the first step of the study entailed acquiring the mass spectra of the various standards using infusion mode, with both ESI and APCI ion sources, in positive and negative ionisation modes. For APCI ionisation, methanol was added to the standard solution in a 9:1 ratio through the use of a T valve and HPLC pump.

Simultaneously, MS^2 experiments were performed, and compound dependant parameters (e.g. collision energy) were optimised accordingly to the "quantitative optimisation" function of the software. The collision energy and the other compound dependent parameters have been chosen to obtain the best sensitivity towards the pseudomolecular ion in SIM detection and the main fragments in MRM detection. Fragmentation experiments (MS) were carried out on the pseudo-molecular ions, both with quadrupole and ion trap (LIT) as analysers, and the corresponding mass spectra were acquired. The fragments obtained for each set up are summarised in Table 2, and the instrumental parameters in Table 3.

Negative detection mode allowed for detecting the highest number of target analytes, thus negative detection was chosen for further optimisation [28]. The second step of the optimisation entailed coupling the MS detector with the HPLC system by analysing standard solutions of selected anthraquinones, flavonoids, benzoic acids and indigoids (5 ppm c.a.). Chromatograms were acquired in full scan, SIM and MRM and are shown in Fig. 1 for ESI and APCI ion sources. The MRMs chosen for ESI and APCI are listed in Table 3. The results obtained for the use of Q3 as ion trap (LIT) or as quadrupole were also evaluated. Details on SIM and MRM scanning modes are given in Table 3.

3.2. Qualitative results

The main peak obtained for all the investigated compounds by infusion analysis is the pseudo-molecular ion. As expected, fragmentation patterns do not significantly change with ionisation source, whereas the relative intensities of peaks differ (see Table 2). ESI interface allows for detecting all the investigated anthraquinones, hydroxybenzoic acids and flavonoids; it fails to detect the indigoids except for indirubin. Notably, it was not possible to obtain any spectrum for carminic acid in positive mode. APCI interface allowed for detecting all the flavonoid compounds both in positive and negative mode whereas anthraquinones were detected in negative mode only. 4-hydroxybenzoic, 2,4- and 3,4-dihydroxybenzoic acids were detected both in positive and negative modes, gallic acid only in negative. Negative mode allowed for the analysis of all of the indigoid compounds under study.

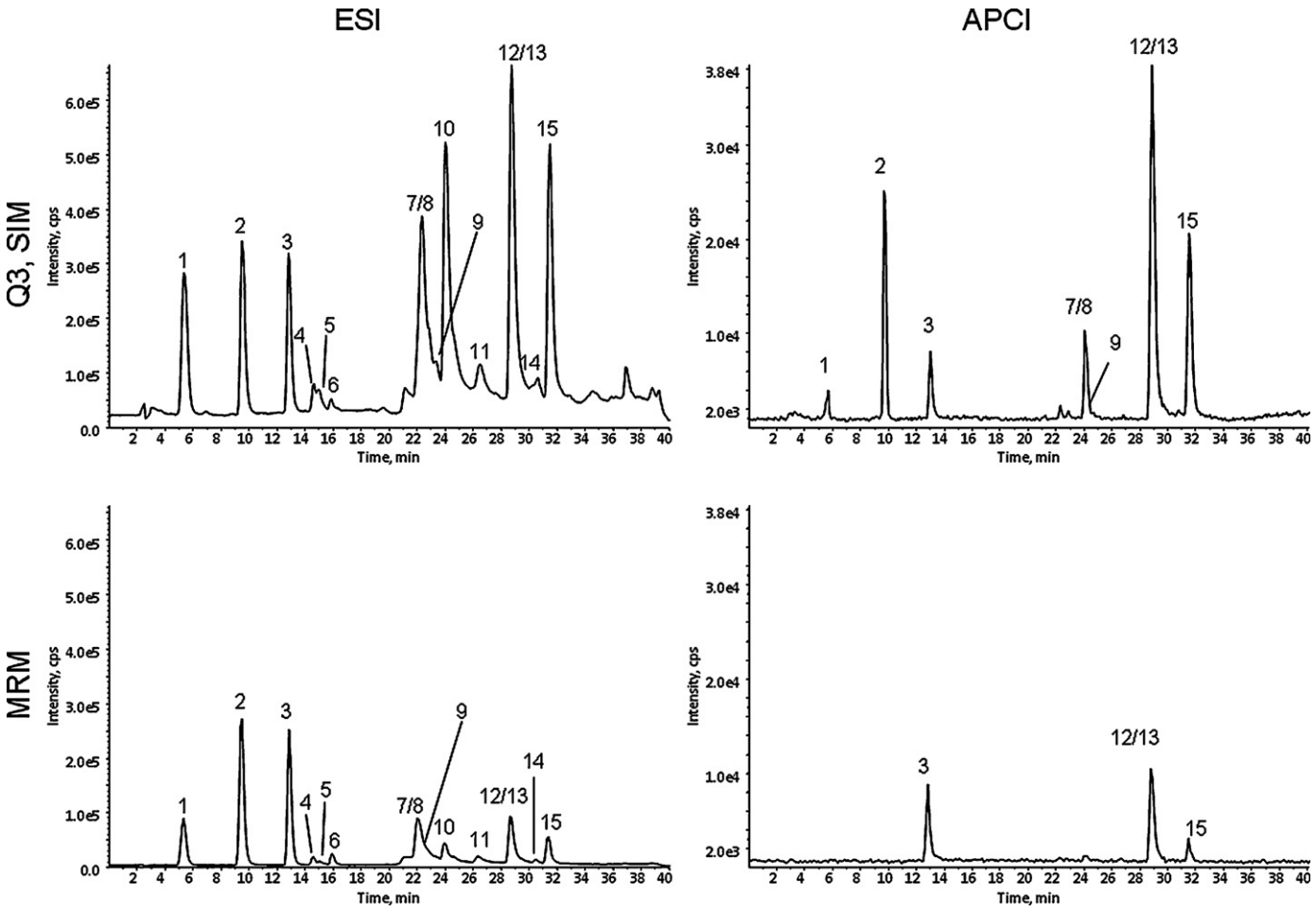

Fig. 1. Chromatograms obtained with different ion sources and MS acquisition methods (negative ions detection) for a standard solution of approximately 2.5×10^{-5} M (1) gallic acid; (2) 3,4-dihydroxybenzoic acid; (3) 4-hydroxybenzoic acid; (4) 2,4-dihydroxybenzoic acid; (5) carminic acid; (6) rutin; (7) quercetin; (8) morin; (9) luteolin; (10) anthragallol; (11) rhamnetin; (12) xanthopurpurin; (13) purpurin; (14) indigotin; (15) emodin; (16) indirubin (not detected); and 5×10^{-5} M and (17) 6,6′-dibromoindigotin (not detected).

It follows a discussion of the fragmentation behaviour of flavonoid, anthraquinoid and hydroxybenzoic compounds in MS^2 experiments:

- The fragmentation of flavonoids, both in positive and negative modes, mainly follows the scheme of a retro Diels–Alder reaction, and subsequently undergoes neutral losses of CO or CO_2. The obtained fragmentation is consistent with literature data [28,29,46–48]: the mechanism has been studied in detail by Ma et al. [49] and by Fabre et al. [50]. MS^n spectra of flavonoids obtained in this work qualitatively agree with the ones described by Hughes et al. [51], by March et al. [52] and by McNab et al. [53] for ESI ionisation and by Justesen [54] for APCI. With regard to flavonoid glycosides, their fragmentation entails the loss of the glycosidic units first, followed by the already discussed fragmentation of the aglycone. Both the glycosides presented in this study – naringin and rutin – loose 309 units, due to the break of the bond between the rutinose and the naringin and quercetin, respectively.
- The fragmentation of anthraquinones is characterised by the loss of 28 units corresponding to the loss of CO and CO_2 [28,47].
- Hydroxybenzoic acids show a very simple fragmentation based on the loss of 44 units, corresponding to the carboxylic group (CO_2) [28].
- Indigoid compounds can be easily recognised by the presence of the molecular ion. It is noteworthy that indirubin was ionised by ESI, while its isomer indigotin was not, in contrast with the literature [42]. This may be ascribed to strong internal hydrogen bonds in the indigotin molecule, which are sterically hindered in the isomer indirubin [46]. Not surprisingly, APCI source allows for ionising all the indigoids [55]. The fragmentation pattern is characterised by the elimination of carbonyl groups [47] and of one of the aromatic rings: the fragment at 156 arise from the elimination of $[C_6H_4COH]$ [31,56].

HPLC–MS analysis confirmed the suitability of ESI interface for anthraquinones, flavonoids and benzoic acids and of APCI for indigoid compounds. ESI ionisation proved more flexible due to its wider range of application; thus ESI was selected as the ionisation source for further optimisation.

3.3. Quantitative results

The pseudo-molecular ion was chosen for SIM acquisition for each analyte. The two main fragment ions obtained for each analyte were chosen for the MRM detection method, in order to enhance the selectivity with respect to possible isomers (e.g. for both luteolin and kaempferol the most intense MRM was 285/151, thus a second MRM was needed for the confirmation of the analyte). For 2,4- and 3,4-dihydroxybenzoic acids, one MRM only shows a suitable intensity. The qualitative results refer to the more intense fragmentation pattern (the first one given in Table 3 for each analyte).

In particular, SIM and MRM acquisition modes were compared in terms of signal-to-noise ratio, relative sensibility and specificity. The average signal-to-noise ratio is around 10^3 for both methods for a concentration of approximately 2.5×10^{-5} M for each analyte. The sensitivity of SIM mode is 10 times higher than the sensitivity of MRM mode for anthraquinones detection, and 5 times higher for the detection of flavonoids and hydroxybenzoic acids. MRM scan allows for distinguishing isomers such as morin and quercetin, while SIM detection does not allow an ultimate confirmation of the structure of the analyte. Concluding, MRM acquisition results to be more suitable in terms of specificity while SIM is more suitable in terms of sensitivity. MRM acquisition was the method of choice (compound dependant parameters for MRM acquisition with ESI and APCI ion sources are reported in Table 3).

Calibration curves were built up for relevant compounds (gallic, 3,4-dihydroxybenzoic, 2,4-dihydroxybenzoic and 4-hydroxybenzoic acids, rhamnetin, quercetin, carminic acid and purpurin) for both UV detection at 254 nm and at 275 nm and for ESI interface with MRM acquisition. Limits of detection and quantitation (LODs and LOQs) were evaluated and are reported in Table 4. Limits of detection and quantitation are calculated as the concentration equivalent to 3 and 10 times the standard deviations (calculated on 6 replicas) of peak area of the most diluted solution, respectively.

Table 4 clearly shows that MRM detection allows for better sensitivity and lower LODs than UV detection at 275 nm and at 254 nm. Moreover, the obtained LODs are of the same order of magnitude than the ones obtained by Surowiec et al. [45] with an optimised DAD detection.

On the one hand, it is interesting to notice that the LODs obtained with MRM detection in this study are comparable to the best LODs obtained with SIM detection and presented in the current literature [28]. On the other hand, it has to be stressed that MRM detection allows for a higher selectivity than SIM detection, due to its capability to distinguish between isomers.

3.4. Aged reference specimens

The developed analytical technique was applied to the analysis of reference specimens subjected to artificial ageing, namely to undyed wool and silk yarn, to alum mordanted wool yarn and to alum mordanted wool yarn dyed with Persian berries (see Section 2).

The chromatograms obtained with LC/MS for the analysis of extracts of not-mordanted wool samples at different ageing times show the increase of the relative amount of hydroxybenzoic acids. Chromatograms obtained for mordanted wool and for silk yarns show a similar trend. In particular, the peak due to 4-hydroxybenzoic acid is clearly visible in all the chromatograms.

Quantitative measurements show an increase in 4-hydroxybenzoic acid in blank woollen and silk yarns with ageing time. The medium-high values of the squared Pearson correlation coefficient R^2 (see Table 5) suggest a linear proportion between the relative amount of 4-hydroxybenzoic acid and ageing time. Nonetheless, the R^2 values (0.91) are not sufficiently high to allow us developing a kinetic predictive model for such complex systems as wool (and silk) yarns. It is interesting to note that the linear coefficient is different for different types of wool, for mordanted and not-mordanted wool and for silk. Particularly, the increase in 4-hydroxybenzoic acid is higher for silk than for wool; it is slightly higher for non mordanted wool than for Al-mordanted one. Moreover, it should be noticed that in the case of silk, standard deviation of measurements is relatively high.

Both 4-hydroxybenzoic and 3,4-dihydroxybenzoic acids are present in Persian berries dyed textiles, and their relative amount increases with time, whereas the amount of chromophore-

Table 4
Calibration curves parameters (a: slope (area μg^{-1} mL); b: intercept (area); R: Pearson coefficient) and limits of detection and quantitation for UV–vis detection (acquisition at 254 nm and at 275 nm) and MS (ESI source, negative ionisation and MRM detection).

Compound	UV at 254 nm					UV at 275 nm					MS (MRM acquisition)				
	Calibration curve parameters			LOD (ng/mL)	LOQ (ng/mL)	Calibration curve parameters			LOD (ng/mL)	LOQ (ng/mL)	Calibration curve parameters			LOD (ng/mL)	LOQ (ng/mL)
	a	b	R^2			a	b	R^2			a	b	R^2		
Gallic acid	6.3×10^4	1.3×10^4	0.9963	223	744	1.2×10^5	0	0.9998	61	203	5.8×10^5	8.1×10^3	0.9994	8.0	26.0
3,4-Dihydroxy benzoic acid	9.1×10^4	1.1×10^4	0.9980	166	554	3.5×10^4	0	0.9989	70	233	1.5×10^5	6.5×10^3	0.9951	10.0	40.0
2,4-Dihydroxy benzoic acid	1.4×10^5	2.2×10^3	0.9996	26	87	4.4×10^4	0	0.9992	30	100	1.5×10^5	5.6×10^2	0.9981	20.0	50.0
4-Hydroxy benzoic acid	2.2×10^5	4.5×10^3	0.9995	50	167	1.0×10^5	0	0.9998	20	67	1.0×10^6	5.0×10^4	0.9984	15.0	49.0
Rhamnetin	7.4×10^4	-1.1×10^3	0.9995	110	367	3.8×10^4	5.4×10^3	0.9996	51	170	1.0×10^5	3.1×10^4	0.9989	5.0	18.0
Quercetin	1.2×10^5	-9.7×10^3	0.9995	47	156	5.5×10^4	3	0.9993	14	47	7.3×10^5	3.2×10^3	0.9995	10.0	41.0
Carminic acid	2.6×10^4	0	0.9937	115	383	9.5×10^4	0	0.9993	10	33	8.9×10^4	3.1×10^3	0.9982	10.0	35.0
Purpurin	1.2×10^5	4.0×10^3	0.9997	31	103	3.9×10^4	0	0.9744	60	200	1.4×10^5	4.2×10^3	0.9972	0.4	1.3

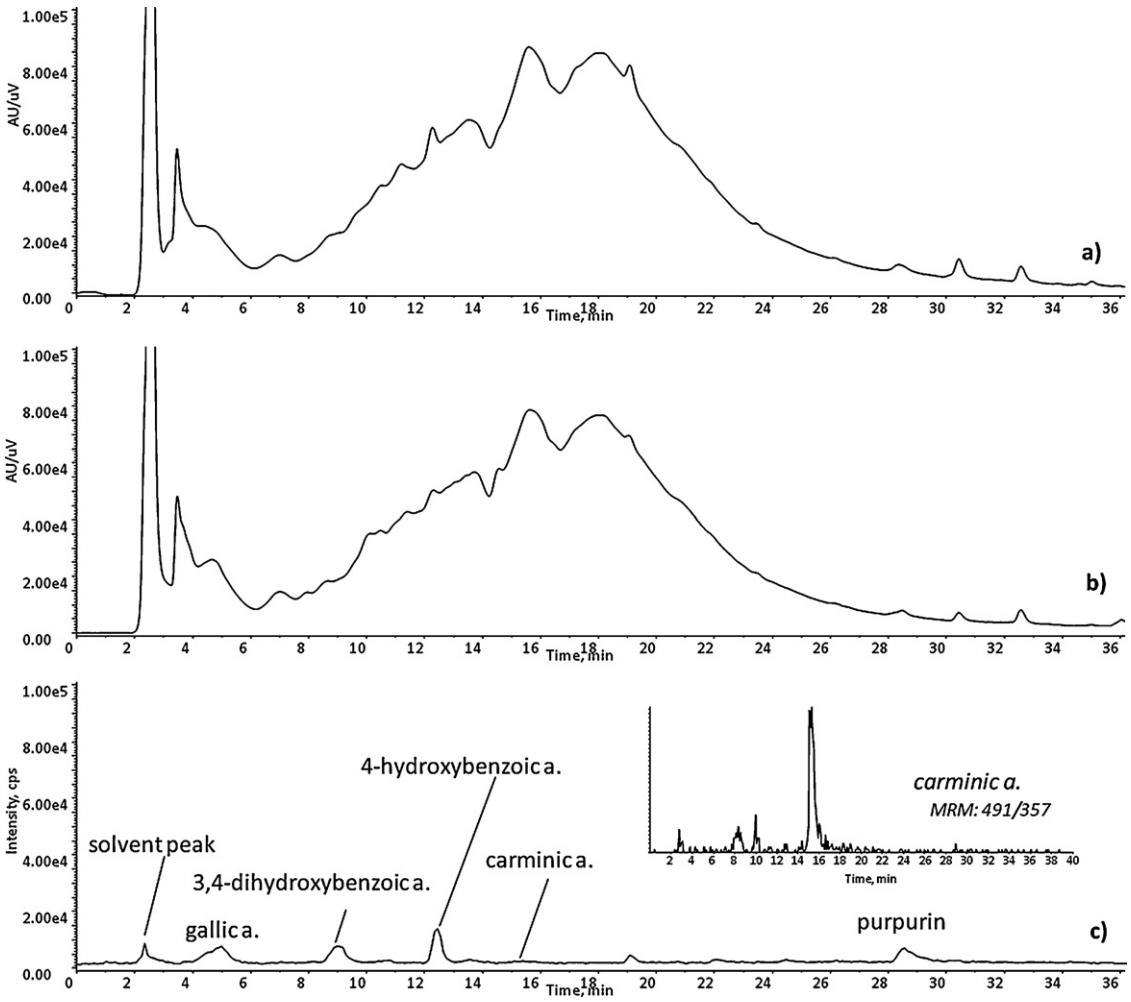

Fig. 2. HPLC-UV–MS chromatogram of sample III33 from The final attack on Jerusalem tapestry, provided by OPD: (a) at 254 nm; (b) at 275 nm; (c) MRM detection. The inset shows the extracted chromatogram for the 491/357 reaction (fragmentation of carminic acid pseudomolecular ion).

containing molecules decreases with ageing time. The presence of isorhamnetin and rhamnazin was also assessed in the TIC of the extract of yarns dyed with Persian berries, but a discussion on their occurrence was not included into the present study due to the lack of proper reference standards.

The literature [36] correlates the occurrence of hydroxybenzoic acids in aged textiles to the photo-induced oxidation of flavonoid dyes; nevertheless their presence in undyed specimens can not be ascribed to the degradation of chromophore-containing molecules. On the one hand, the degradation of quercetin and rhamnetin may explain the occurrence and the relative increase of 3,4-dihydroxybenzoic acid in the case of Persian berries dyed textiles. On the other hand, the photo-oxidation of quercetin and rhamnetin can not account for the occurrence of 4-hydroxybenzoic acid and its relative increase with ageing.

Thus, the detection of 4-hydroxybenzoic acid in undyed and dyed specimens is particularly interesting; in order to rationalise the reaction pathway leading to this compound, some hypothesis were taken into account. Its presence in undyed samples suggests that 4-hydroxybenzoic acid can be the product of the degradation of specie originally present in the matrix.

On the basis of the literature data [18–23], 4-hydroxybenzoic acid is most probably formed from tyrosine or tryptophan due to a photo-induced reaction where oxygen attacks the reactive benzylic carbon atom.

The evaluation of the increase in 4-hydroxybenzoic acid in undyed specimens gave interesting results. It is not surprising that the rate of reaction differs depending on the kind of material analysed. The linear coefficient for silk is almost 10 times bigger than the one for wool; this finding is consistent with literature data: silk is known to be much more prone to photo-yellowing and photo-tendering then wool. The differences among different kinds of wool and mordanted and not-mordanted wool are not significant.

This study confirms the presence of 4-hydroxybenzoic acid in yarns dyed with Persian berries (hydroxybenzoic acids are known precursors of flavonoids and other plants bioactive molecules in

Table 5
Parameters describing the linear dependence of the 4-hydroxybenzoic acid relative amount ($\mu g\,mL^{-1}$) vs. the time of ageing (h) in reference not dyed specimens extracts.

Sample	Slope ($\mu g\,mL^{-1}\,h^{-1}$)	Std. dev. slope (RSD)	Intercept ($\mu g\,mL^{-1}$)	Std. Dev. int (RSD)	n	R^2
W_a, purchased from a craftsman in Poland	2.8×10^{-3}	5.3×10^{-8} (0.002%)	-4.9×10^{-1}	1.1×10^{-2} (2.173%)	17	0.9106
W_a, Al mordanted	3.1×10^{-3}	6.9×10^{-8} (0.002%)	-5.4×10^{-1}	1.4×10^{-2} (2.545%)	17	0.9006
W_b, "lana Campolmi"	1.8×10^{-4}	5.3×10^{-11} (0.000%)	-6.5×10^{-3}	4.06×10^{-6} (0.061%)	14	0.9816
Silk ("seta Schappe" provided by OPD)	9.8×10^{-3}	5.6×10^{-7} (0.006%)	−1.4	1.1×10^{-1} (7.590%)	18	0.9149

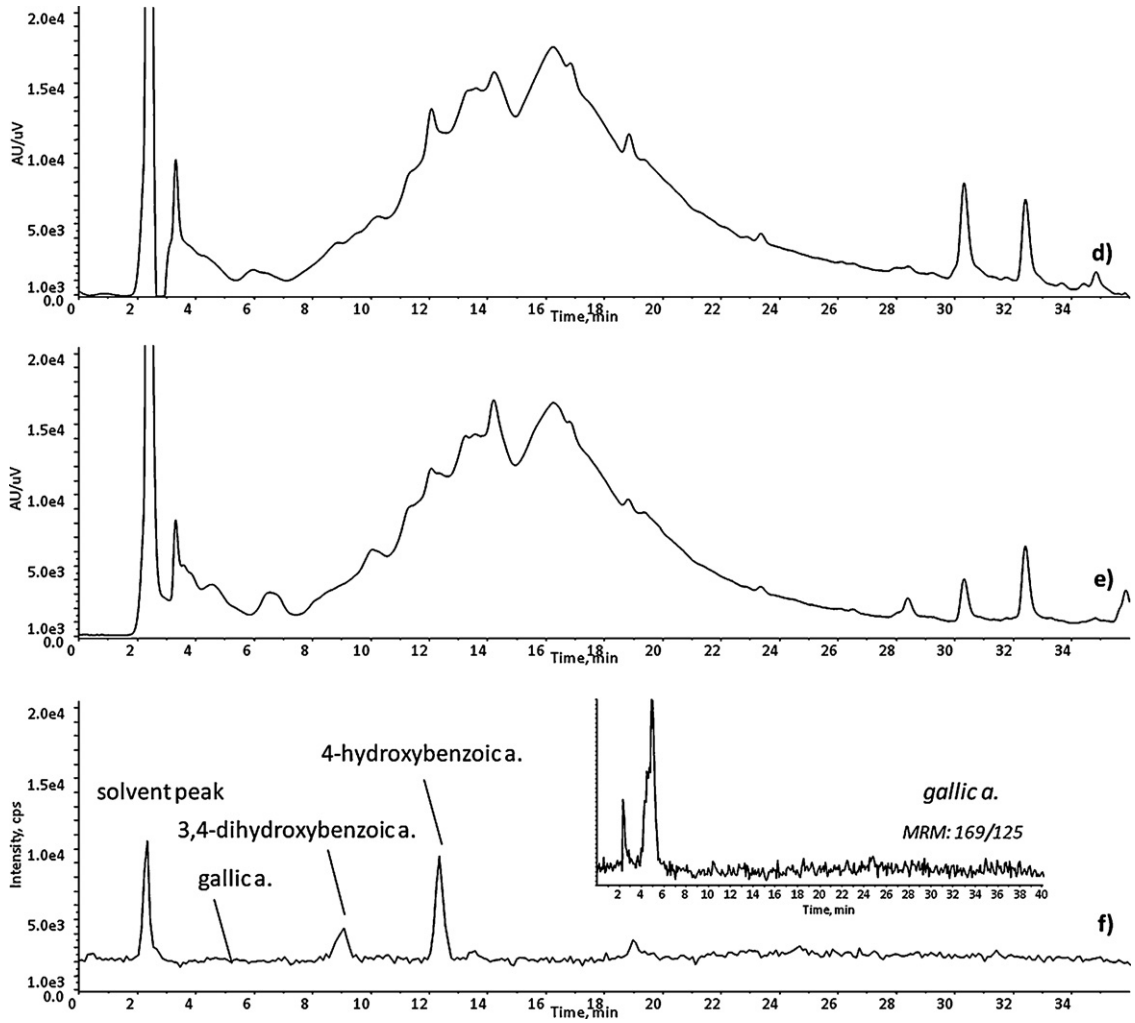

Fig. 3. HPLC-UV–MS chromatogram of sample IIIH′ from The final attack on Jerusalem tapestry, provided by OPD: (d) at 254 nm; (e) at 275 nm; (f) MRM detection. The inset shows the extracted chromatogram for the 169/125 reaction (fragmentation of gallic acid pseudomolecular ion).

plants, and were detected in the extracts of raw materials). Moreover, the analytical results show that 3,4-dihydroxybenzoic acid can not be considered as a molecular marker for ageing of flavonoid dyes, as suggested in the literature, due to its presence in the freshly dyed specimens [36].

With regard to dyed specimens, chromatographic profiles change with ageing and the relative amount of hydroxybenzoic acids increases while the relative amount of chromophore-containing molecules decreases, in agreement with the literature [36,10,11,14]. Nevertheless, it is difficult to devise a fitting model to explain the overall process, due to the fact that hydroxybenzoic acids are originally present in the freshly dyed yarns and can also be produced by the photo-oxidation of both the chromophore-containing molecules and the matrix.

It can be concluded that the presence of 4-hydroxybenzoic acid in yarn extracts is a strong indication of ageing, whereas the detection of di- and tri-hydroxybenzoic acids does not imply that the textile was subjected to ageing.

4. Case study

The developed analytical technique was applied to the characterisation of selected samples from a medieval tapestry, *The final attack on Jerusalem*. The results are presented in Table 6, and the chromatograms obtained for two explicative samples are shown in Figs. 2 and 3.

The results highlighted that HPLC–MS technique detected chromophore-containing molecules in the samples extracts at sub-ppm concentrations, thus allowing for identifying all the minor components of extremely complex mixtures of dyes, which could not be detected by classical methods such as HPLC–UV, as can be clearly seen in Figs. 2 and 3.

Red dyes contained alizarin and purpurin, known molecular markers of madder-type dyes.

The black samples were dyed with two different techniques. The much degraded yarns were dyed with a tannin based dye, extracted from gallnuts, alder bark or sumac (molecular marker: gallic acid). The colour in the threads in good condition was achieved by superimposing colours (*colour cupo* in ancient recipes books), most probably with cochineal (molecular marker: carminic acid), a madder-type dye (purpurin) and probably weld (luteolin, apigenin) and indigoid dye baths, and eventually by adding gallo-tannins as well (gallic acid). Unfortunately, the developed procedure did not allow for detecting minor dye components that could be used for a decisive dye identification.

Thanks to the use of the UV detection, also indigotin was detected in the blue yarns, thus suggesting the use of an indigoid dye.

Table 6
Results of the HPLC-DAD-ESI–MS analysis of samples collected from The final attack on Jerusalem tapestry.

Sample *n*	Colour	Identified compounds
IV m′	Blue	Indigotin
IV s	Red	Alizarin, purpurin
III H′	Dark brown	Gallic a. + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.
I 28	Black	Carminic a. + luteolin + apigenin + gallic a. + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.
I 29	Black	Carminic a. + purpurin + luteolin + apigenin + indigotin (traces) + gallic a. + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.
III 19	Dark brown	gallic a. + 4-hydroxybenzoic a.
III 33	Black	Carminic a. + purpurin + gallic a. + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.
III 40	Black	Carminic a. + purpurin + indigotin (traces) + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.
I Q*	Black	Carminic a. + luteolin + apigenin + gallic a. + 3,4-dihydroxybenzoic a. + 4-hydroxybenzoic a.

The comparison between visual inspection of samples and analytical results suggests that the original yarns were dyed with gallo-tannins with an iron mordant and quickly degraded [3]. In a short time, the tapestry was restored by using different yarns, dyed by superimposing colours, with an alum mordant.

It is very important to notice that all the analysed yarns contained 4-hydroxybenzoic acid in high amounts, thus confirming its suitability as a marker of ageing of the target samples.

5. Conclusions

The analysis of samples collected from works of art is an extreme challenge for the reduced size of the collected material, for the low abundance of target compounds and for the presence of ageing products.

The proposed procedure proved to be extremely sensitive and selective, with limits of detection between 0.4 and 20 ng/mL and a very high specificity towards the target analytes, thanks to the optimisation of the mass spectrometric parameters. Moreover, the use of MRM detection allows for the ultimate confirmation of the analyte identity and thus resulted to be more satisfactory than the currently used SIM acquisition.

On the one hand, the proposed method fails to detect indigotin, the molecular marker of indigoid dyes. On the other hand, indigotin can be easily detected by the on-line UV detector.

In this study, the analytical procedure was also exploited to study and understand ageing processes occurring in historical dyed materials, with particular attention to the degradation of the textile matrix. Aged reference materials were prepared and subjected to HPLC–ESI-MS analysis. The known photo-oxidative degradation products of flavonoid dyes were identified and quantified in the aged materials. More interestingly, 4-hydroxybenzoic acid was identified and the increase in its relative amount in the yarns with time was assessed. This evidence shows that 4-hydroxybenzoic acid is a degradation product of the proteinaceous matrix (wool or silk), most probably originating from the oxidation of tyrosine. Moreover, the analytical results show that 3,4-dihydroxybenzoic acid can not be considered as a molecular marker for ageing of flavonoid dyes, as suggested in the literature, due to its presence in the freshly dyed specimens.

The ability of the proposed procedure to detect the molecular markers of dyeing materials was tested on samples collected from a medieval tapestry. The known molecular markers of madder-type and indigoid dyes were detected in relevant amounts in the red and blue yarns, respectively. The developed procedure presents some limitations in identifying minor dye components that could be used for a decisive dye identification; nevertheless its ability to detect the chromophore-containing molecules in very low amounts proved fundamental in identifying the molecular markers in black yarns dyed by superimposing colours. The analytical results helped the restorers in understanding the dyeing procedures used, thus allowing them to choose the best restoration procedure.

Acknowledgments

The authors gratefully acknowledge the staff of the tapestry section of the Opificio delle Pietre Dure (Florence) for providing the samples described in the case study and for the fruitful discussion of the results by a conservation point of view. PRIN 2007 is acknowledged for the financial support.

The authors would like to thank the Structural Research Laboratory (SRL) at the Department of Chemistry of University of Warsaw for using HPLC–MS. SRL has been established with financial support from European Regional Development Found in the Sectorial Operational Programme “Improvement of the competitiveness of Enterprises, years 2004–2005” project no: WPK_1/1.4.3./1/2004/72/72/165/2005/U.

References

[1] D. Cardon, Le monde des teintures naturelles, Editions Bellin, Paris, 2003.
[2] F. Brunello, L’arte della tintura nella storia dell’umanità, Neri Pozza Ed, Vicenza, 1968.
[3] J.H. Hofenk de Graaff, The Colourful Past, Origins Chemistry and Identification of Natural Dyestuffs, Abegg-Stiftung/Archetype Publications Ltd., London/Riggisberg, 2004.
[4] P. Cox Crews, J. Am. Inst. Conserv. 21 (1982) 43.
[5] T. Padfield, S. Landi, Stud. Conserv. 11 (1966) 181.
[6] C.H. Giles, D.J. Walsh, R.S. Sinclair, J. Soc. Dyers Colour (1977) 348.
[7] N. Kohara, C. Sano, H. Ikuno, Y. Magoshi, M.A. Becker, M. Yatagai, M. Saito, in: J.M. Cardamone, M.T. Baker (Eds.), Historic Textiles, Papers, and Polymers in Museums, The American Chemical Society, 2001, p. 74.
[8] M. Yatagai, Y. Magoshi, M.A. Becker, C. Sano, H. Ikuno, N. Kohara, M. Saito, in: J.M. Cardamone, M.T. Baker (Eds.), Historic Textiles, Papers, and Polymers in Museums, The American Chemical Society, 2001, p. 86.
[9] I. Joosten, M.R. van Bommel, R. Hofmann-de Keijzer, H. Reschreiter, Microchim. Acta 155 (2006) 169.
[10] A. Quye, J. Wouters, J.J. Boon, ICOM Committee for Conservation, Scotland, 1–6 September 1996: preprins, Vol II, 1996, p. 704.
[11] E.S.B. Ferreira, A. Quye, H. McNab, A.N. Hulme, J. Wouters, J.J. Boon, ICOM Committee for Conservation, 12th Meeting, Vol I, 1999, p. 221.
[12] D.A. Peggie, A.N. Hulme, H. McNab, A. Quye, Microchim. Acta 162 (2008) 371.
[13] E.S.B. Ferreira, New approaches towards the identification of yellow dyes in ancient textiles, PhD Thesis, The University of Edimburgh, 2002.
[14] E.S.B. Ferreira, A. Quye, H. McNab, A. Hulme, Dyes Hist. Archaeol. 19 (2003) 13.
[15] X. Zhang, R. Boytner, J.L. Cabrera, R. Laursen, Anal. Chem. 79 (2007) 1575.
[16] C. Ahn, S.K. Obendorf, Text. Res. J. 74 (2004) 949.
[17] C. Clementi, W. Nowik, A. Romani, F. Cibin, G. Favaro, Anal. Chim. Acta 596 (2007) 46.
[18] I. Vanden Berghe, J. Wouters, in: R. Janaway, P. Wyeth (Eds.), Scientific Analysis of Ancient and Historic Textiles: Informing Preservation, Display and Interpretation, Archetype, London, 2005, p. 151.
[19] M. Odlyha, C. Theodorakopoulos, R. Campana, AUTEX Res. J. 7 (2007) 9.
[20] J.S. Church, K.R. Millington, Biospectroscopy 2 (1996) 249.
[21] K.R. Millington, J.S. Church, J. Photochem. Photobiol. B 39 (1997) 204.
[22] R.S. Davidson, J. Photochem. Photobiol. B 33 (1996) 3.
[23] J.M. Dyer, S.D. Bringans, W.G. Bryson, Photochem. Photobiol. Sci. 5 (2006) 698.
[24] I. Degano, E. Ribechini, F. Modugno, M.P. Colombini, Appl. Spectrosc. Rev. 44 (2009) 363.
[25] J. Wouters, Stud. Conserv. 30 (1985) 119.
[26] Z.C Koren, Dyes Hist. Archaeol. 13 (1994) 27.
[27] I. Petroviciu, F. Albu, A. Medvedovicic, Microchem. J. 95 (2010) 247.
[28] I. Surowiec, B. Szostek, M. Trojanowicz, J. Sep. Sci. 30 (2007) 2070.
[29] L. Rafaëlly, S. Héron, W. Nowik, A. Tchapla, Dyes Pigments 77 (2008) 191.
[30] I. Karapanagiotis, Y. Chryssoulakis, Ann. Chim. 96 (2005) 75.
[31] M. Puchalska, K. Polec-Pawlak, I. Zadrozna, H. Hryszko, M. Jarosz, J. Mass Spectrom. 39 (2004) 1441.

[32] K. Pawlac, M. Puchalska, A. Miszczak, E. Rosloniec, M. Jarosz, J. Mass Spectrom. 41 (2006) 613.
[33] R. Marques, M.M. Sousa, M.C. Oliveira, M.J. Melo, J. Chromatogr. A 1216 (2009) 1395.
[34] X. Zhang, R.A. Laursen, Anal. Chem. 77 (2005) 2022.
[35] X. Zhang, R.A. Laursen, J. Mass Spectrom. 284 (2009) 108.
[36] E.S.B. Ferreira, A. Quye, H. McNab, A. Hulme, Dyes Hist. Archaeol. 18 (2002) 63.
[37] L. Valianou, K. Stathopoulou, I. Karapanagiotis, P. Magiatis, E. Pavlidou, A.L. Skaltsounis, Y. Chryssoulakis, Anal. Bioanal. Chem. 394 (2009) 871.
[38] L. Valianou, I. Karapanagiotis, Y. Chryssoulakis, Anal. Bioanal. Chem. 395 (2009) 2175.
[39] M.R. van Bommel, Dyes Hist. Archaeol. 20 (2005) 30.
[40] J. Orska-Gawrys, I. Surowiec, M. Biesaga, M. Hutta, R. Halko, K. Urbaniak-Walczak, M. Trojanowicz, Anal. Lett. 36 (2003) 1211.
[41] M.P. Colombini, A. Carmignani, F. Modugno, F. Frezzato, A. Olchini, H. Brecoulaki, V. Vassilopoulou, P. Karkanas, Talanta 63 (2004) 839.
[42] M.A. Ackacha, K. Polec-Pawlak, M. Jarosz, J. Sep. Sci. 26 (2003) 1024.
[43] E.S.B. Ferreira, A. Quye, H. McNab, A.N. Hulme, J. Wouters, J.J. Boon, Dyes Hist. Archaeol. 16 (17) (2001) 179.
[44] M.P. Colombini, A. Andreotti, C. Baraldi, I. Degano, J.J. Lucejko, Microchem. J. 85 (2007) 174.
[45] I. Surowiec, W. Nowik, M. Trojanowicz, Microchim. Acta 162 (2008) 393.
[46] E. Rosenberg, Anal. Bioanal. Chem. 391 (2008) 33.
[47] B. Szostek, J. Orska-Gawrys, I. Surowiec, M. Trojanowicz, J. Chromatogr. A 1012 (2003) 179.
[48] M. Ye, J. Han, H. Chen, J. Zheng, D. Guo, J. Am. Soc. Mass. Spectrom. 18 (2007) 82.
[49] Y.L. Ma, Q.M. Li, H. Van den Heuvel, M. Claeys, Rapid Commun. Mass Spectrom. 11 (1997) 1357.
[50] N. Fabre, I. Rustan, E. deHoffmann, J. Quetin-Leclercq, J. Am. Soc. Mass. Spectrom. 12 (2001) 707.
[51] R.J. Hughes, T.R. Croley, C.D. Metcalfe, R.E. March, Int. J. Mass Spectrom. 210 (211) (2001) 371.
[52] R.E. March, X.S. Miao, Int. J. Mass Spectrom. 231 (2004) 157.
[53] H. McNab, E.S.B. Ferreira, A.N. Hulme, A. Quye, Int. J. Mass Spectrom. 284 (2009) 57.
[54] U. Justesen, J. Chromatogr. A 902 (2000) 369.
[55] I. Karapanagiotis, V. Villermereuil, P. Magiatis, P. Polychronopoulos, K. Vougogiannopoulou, A.-L. Skaltsounis, J. Liq. Chromatogr. 29 (2006) 1491.
[56] K. Polec-Pawlak, M. Puchalska, J. Witowska-Jarosz, M. Jarosz, in: L. Meijer, N. Guyard, L. Skaltsounis, G. Eisenbrand (Eds.), Indirubin, The red Shade of Indigo, Life in Progress Editions, Roscoff, France, 2006, p. 115.