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Analytical methods for determination of anthraquinone dyes in historical textiles: A review

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Abstract

Historical and archaeological textiles are among the most crucial and vulnerable records of our social and cultural history. Analysis of organic colorants found in these materials is unquestionably one of the most powerful tools to understand historical developments, cultural exchanges, and progress in science and technology. Natural anthraquinones represent the most commonly used natural colorants for textile dyeing until the late 19th century. The identification of anthraquinones in cultural heritage objects is a challenging task due to the small size of historical samples, diversity of potential dye sources, variable extraction procedures and dyeing methods, complex chemical constitution, structurally analogous chromophores, and possible presence of degradation products and contaminants. Developments in dye analysis of historical interest have originated and expanded along with the general advances in analytical science. In the last few decades, a close cooperation between science and cultural heritage disciplines contributed enormously to this field. The topic of historical dyes and their analysis in textiles, artworks, archaeological objects and cultural heritage materials has been reviewed several times in the last fifteen years. However, no review has been published to-date exclusively on the analysis of anthraquinone colorants in historical and archaeological textiles. Overall, liquid chromatography (LC)-based techniques have been the most widely used method for anthraquinone dye analysis. Owing to increasing demand of minimally invasive/non-invasive techniques, recent developments of novel techniques have resulted in the availability of many alternative/complementary methods to LC-based analysis. This review begins with a short overview of sources, chemistry and importance of natural anthraquinone dyes found in historical textiles before turning to a detailed discussion on developments involving established and emerging analytical techniques of anthraquinone dye analysis for textile cultural heritage materials. To illustrate the state-of-the-art, representative examples of analytical techniques highlighting their advantages, limitations and applicability are also presented.

Keywords: Anthraquinone dyes; Historical dye analysis; High performance liquid chromatography; Surface enhanced Raman spectroscopy; Fiber optics reflectance spectroscopy; Mass spectrometry

Contents

1. Introduction

Organic colorants extracted from a variety of natural sources have been used since antiquity for textile dyeing purposes [1-3]. The earliest evidence so far of coloured fibres are 30,000-year old wild flax remains from archaeological finds in Upper Palaeolithic layers at Dzudzuana Cave, Georgia, although the identity of colouring materials was not confirmed [4] and indigoid dyes have been identified analytically in a 6,200-year-old Peruvian textile [5]. The oldest scientifically-proven evidence for anthraquinones as madder for dyeing are Bronze Age textiles dated 3,000 to 3,200 years old from theYanghai archaeological site in the Xinjiang Region, China [6]. Considering the cultural significance and complex spectrum of our textile legacy dating back to the prehistoric era, researchers from universities, museums and cultural heritage institutions are searching for innovative methodologies to interpret and safeguard our textile heritage through surviving material evidence. In recent decades, the elucidation of dyes in historical and archaeological textiles has gained increasing attention in the fields of cultural heritage and conservation practices [7] with dye analysis becoming one of the most interpretative and reliable approaches for understanding the societal and scientific developments in various cultures and historical periods. The detection and identification of colorants present in textiles of archaeological or historical importance sheds unprecedented light on social, cultural and economic status of a particular period or geographical area, its trade connections, and technological capabilities of its population. The unambiguous characterization of dyes is of immense value to the historians, archaeologists and conservators addressing questions on the provenance, authentication and restoration of our irreplaceable textile heritage, with the data from dye analysis being important for factual documentation, deterioration assessment and rational choice of conservation treatments and restoration of historical artefacts. The chemical characterization of dyes in heritage textile objects is also

relevant to preventive conservation strategies and long-term preservation as many organic colorants are not stable to light over long periods of exposure[8].

Anthraquinones are typically present in the most common red natural colorants used extensively in textile dyeing, from many thousand years ago until the late 19th century [9-12]. The identification of anthraquinones in cultural heritage objects is a challenging task and requires highly selective, sensitive and reliable analytical methods due to the small size of historical samples, diverse potential dye sources, variable extraction procedure and dyeing methods, complex chemical constitution, structurally analogous chromophores and possible presence of degradation products and contaminants. In 1985, Wouters [13] showed how liquid chromatography with UV-Vis wavelength detection could be applied as a quantitative analytical technique to gain a clearer insight into the natural colorant sources for historical dyeing and the effects of environmental conditions on their preservation. In the last few decades, a close cooperation between science and cultural heritage disciplines has contributed enormously to the emergence and rapid development of several micro-destructive and non-destructive techniques enabling source identifications and characterization of anthraquinone dyes in historical textiles and other materials of cultural significance. Heritage science researchers are now equipped with highly sensitive microanalytical techniques to routinely detect and identify these chromophores and associated degradation products in complex historical specimens at nanogram levels. Currently, high performance liquid chromatography (HPLC) coupled with suitable detectors stands out as the most reliable and versatile technique for organic colorant analysis in historical specimens [14, 15]. More recently, developments in direct analysis for real time-mass spectroscopy (DART-MS)[16, 17], surface-enhanced Raman spectroscopy (SERS) [8, 18], fiber-optics reflectance spectroscopy (FORS)[19] and fluorescence spectroscopy [20] have given heritage scientists access to a range of complementary analytical techniques.

Chemical analysis of dyes of historical interest is an evolving field. The topic of historical dyes and their analysis in textiles, artworks, archaeological objects and cultural heritage materials [1, 7, 8, 14, 15, 18, 21-23] has been reviewed several times in the last fifteen years. This review will focus on the identification of natural anthraquinone dyes in historical and archaeological textiles. We begin with a short overview of sources, chemistry and importance of natural anthraquinone dyes found in historical textiles before turning to a detailed discussion on developments involving established and emerging analytical techniques and practical know-how of anthraquinone colorant characterization in cultural heritage textile materials. To illustrate the state-of-the-art, representative examples of analytical techniques highlighting their advantages, limitations and applicability are also presented.

2. Anthraquinone dyes in historical textiles

Naturally occurring anthraquinones constitute the largest group of natural quinoids based on a 9,10-anthraquinone skeleton **1** (Fig. 1), and typically occur in their glycosidic forms in nature [24, 25]. Although the specific pathway of anthraquinone biosynthesis is still a matter of debate, and a variety of different precursors and pathways have been proposed, the established literature indicates that shikimate (chrosimate/o-succinylbenzoic acid) and polyketide (acetate-malonate) pathways are the main biosynthetic routes leading to anthraquinone formation in many plants and some insects, respectively (Fig. 1) [27, 28]. Natural dyes applied without additives or dyeing agents often result in narrow shade range and lower color fastness of the dyed textiles [26]. Tinctorial properties can be greatly improved with dyeing agents called mordants. Mordants are metal salts or other auxiliary substances with affinity for both the dye and the fibres that enhance dye uptake and fastness properties of the resulting dyed textile. Differences in the dye-mordant-fibre complex formed mean that mordants may darken, brighten or change the final color of the dyed fibre. Natural anthraquinones extracted from certain plant roots and scale insects produce a beautiful colour

palette of red hues on different types of fibre, with a range extending to orange, purple and brown depending on the metallic salt used for the mordant [2, 29].The most celebrated sources of red natural dyes were the roots of plants belonging to *Rubiaceae* family (e.g. madder, chay root, Indian mulberry) and scale insects (e.g. cochineal, kermes, lac) [1]. These natural anthraquinone dyes enjoyed commercial success around the world over many centuries, until their market started collapsing with the development of the first artificial anthraquinone dye, alizarin, in 1868 [30]. Some most commonly detected anthraquinone dyes in historical textiles are listed in Fig. 2 and their sources are given in Table 1 [1, 13, 31-34].

2.1 Plant anthraquinones

The most common sources of red anthraquinone dyes throughout history were the roots of *Rubiaceae* family plants, in particular madders (*Rubia* spp.) which have been admired for their vibrant mordanted red hues on textiles, with evidence of this plant species' use by ancient Indians, Egyptians, Greeks, and Romans[35, 36]. Anthraquinones associated with madder dyestuffs have been detected in historic and archaeological textiles from all over the world, substantiating the extensive use and popularity of this type of plant dye throughout history in different geographical regions. Among the 78 species of *Rubia* genus currently recorded in The Plant List [37], only a few found extensive commercial application as dyestuffs because of their natural abundance, ease of naturalisation, colorant content and colour quality - dyer's madder (*Rubia tinctorium*), wild madder (*Rubia peregrina*), and Indian madder (*Rubia cordifolia*). Although the coloring components and tinctorial properties of these species of *Rubia* differ significantly, the term 'madder' has been used historically to indicate colorants extracted from them all [38, 39]. Out of 68 anthraquinoid compounds detected in madder roots of the various species [40], only a few have been identified in madder-dyed historical textiles [32, 38, 41, 42]. Alizarin **2** and purpurin **3** are the main chromophores in madder dyed textiles, with xanthopurpurin **4**, pseudopurpurin **5**, rubiadin **6**,

munjistin **7**, anthragallol **8**, lucidin **9**, quinizarin **10** and anthraquinone glycosides also present depending on the dyestuff source. Other commonly identified sources of anthraquinone dyes in historical textiles include lady's bedstraw (*Galium verum*), Indian mulberry/Noni (*Morinda citrifolia*), chayroot (*Oldenlandia umbellata*) and *Relbunium* spp [1, 16, 31, 43-46]. Morindone **11**, derived from the hydrolysis of morindin, is the marker compound found in textiles dyed with *Morinda* spp [43, 44, 47]. The roots of *Oldenlandia umbellata* contain alizarin **2** as the major dyeing component with purpurin, xanthopurpurin and ruberythric acid in minor quantities [31, 48, 49]. Purpurin **3**, xanthopurpurin **4**, pseudopurpurin **5**, and munjistin **6** are the main colorants in the roots of *Relbunium* plants [45].

2.2 Insect anthraquinones

Another class of historically important red anthraquinoid dye source were insects belonging to the *Coccideae* family, such as American/Mexican cochineal (*Dactylopius coccus* Costa), Armenian/Ararat cochineal (*Porphyrophora hamelii* Brandt), Polish cochineal/kermes (*Porphyrophora polonica* L.), kermes (*Kermes vermilio* Planchon) and lac (*Kerria lacca* Kerr) [1, 50-53]. Carminic acid **12** is the main coloring component of cochineals, and varying amounts of the relatively minor components kermesic acid **13**, flavokermesic acid **14**, dcII (2-C-glucoside of flavokermesic acid) **20**, dcIV (α-C-glucofuranoside of kermesic acid) **21** and dcVII (β-C-glucofuranoside of kermesic acid) **22** are characteristic for different scale insect species in historical textiles [50, 51, 54]. The main dye components of kermes are kermesic acid **13** and flavokermesic acid **14** [54, 55], whereas those of lac are laccaic acids **14-18**, and erythrolaccin **19** [56-59].

3. Analytical identification of natural anthraquinone dyes

Developments in dye analysis for textiles of historical interest have progressed alongside general advances in analytical science. The breadth and scope of historical dye analysis is rapidly expanding through interdisciplinary connections among researchers from chemistry,

physics, material sciences, archaeology andheritage science, including technical art history and conservation science. Several current methods for dye analysis of cultural heritage objects are based on the identification of characteristic marker components, developed as protocols to compare unknown components in the historical sample with known components in reference dyed textiles prepared by dyeing methods as modern recreations of past methods from historical literature and archival documentation. With historically informed selection of dyeing materials which might have been used in dyeing of historical textiles recreations are performed, often followed by artificial ageing, in order to identify the marker components and possible degradation products. The earliest historical dye analysis methods included identification through extractions and chemical reactions, visual colour matching, paper chromatography, UV-Vis and FTIR spectroscopy [45, 60-63]. Saltzman [64] employed UV–Vis spectroscopy on solutions of dyes extracted from fibre samples of Paracas textiles and identified cochineal and *Relbunium* as anthraquinone dye sources by comparison with spectra of reference dye solutions (Fig. 3). UV-Vis spectroscopy combined with a magnesium acetate colour test positively identified madder and kermes in Anglo- Scandinavian textiles; however, the weak absorption spectra did not allow an unequivocal distinction between madder and kermes [62]. A similar approach was used in another study to identify *Rubia tinctorum*, *Gallium* spp., cochineal and kermes as sources of anthraquinone dyes in Iron Age textiles from Norway and Denmark [65]. The application of thin-layer chromatography (TLC) further improved analytical capability for identification of colouring compounds in historical objects detected by their intrinsic UV absorbance and/or reaction with specific colorimetric reagents [65-68]. TLC has been used for extensive studies of anthraquinone dyestuffs, because of its methodological simplicity and low cost, and a skilled and experienced analyst is key to its successful application [31]. TLC enables separation of dyes, but low separation efficiency, poor automation and lack of quantitative analysis limits its

applicability for researching the chemical complexity of historical dyes. Higher resolution separation methods for historical dye analysis awaited later developments in high performance liquid chromatography (HPLC). HPLC with (photo)-diode array detection (DAD/PDA), fluorescence detection (FLD) or mass spectrometric (MS) detection has the advantage of resolving complex mixtures of related compounds, and thus provides highly-sensitive and reliable methods for characterisation of organic dyestuffs present in historical and archaeological textiles. A few researchers also employed gas chromatography and capillary electrophoresis methods for separation and identification of these dyes [69-71]. All of these techniques are invasive and require the removal of micro-samples from the historical objects. Due to limited availability of historical samples and presence of extremely low amount of dyes in those textiles, the success of the analysis strongly depends on the amount of dye recovered [7, 23]. In the past few decades, development of new analytical techniques has been focused on non-invasive or non-destructive approaches using spectrometric techniques and direct mass spectrometric methods.

For all the techniques applied to dyed historical textiles, identification of the originating dye source depends heavily on reliable, well-characterised reference substances or library [7] and is complicated when material degradation, such as ageing, reduces the amount of original dye compounds and/or introduces degradation products.

3.1 Non-invasive techniques based on reflectance and fluorescence spectroscopy

Although micro-destructive techniques are more informative, an ideal technique for historical textile dye analysis would be non-invasive which allows *in-situ* measurements without affecting the valuable cultural heritage artefact. In this context, application of non-invasive UV-Vis-NIR reflectance spectrometry and excitation-emission matrix (EEM) fluorescence spectroscopy-based techniques have been found useful for the *in-situ* detection of anthraquinone dyes in historical textiles [72, 73]. Although these methods are less specific

than micro-destructive analysis, an *in-situ* survey of materials enables preliminary investigation for diagnostic purposes, or even in certain cases, positive identification of dyestuff without any damage to the historical object.

EEM fluorescence spectrometry, which measures the emission spectra over a wide range of excitation wavelengths, offers higher sensitivity and selectivity with respect to absorption spectrometry [73]. Use of fluorescence spectroscopy is a recent development and is especially advantageous as a non-invasive tool to detect dyes with relatively good emission quantum yield [74]. Anthraquinone colorants with fluorescence properties are widespread in nature, absorbing around 500 nm and emittingover a spectral region of 550 nm to 650 nm relating to the number and position of hydroxyl groups as well as surrounding environment [20]. Investigations on model samples of painting lakes have demonstrated that fluorescence can identify anthraquinone dyes and even distinguish those of animal or vegetal origin [75]. Although several fluorescence-based techniques and approaches have been applied to organic dyes in artworks [20], their application to dyed historical textiles is scarce. Nakamura et al. [73] demonstrated use of EEM fluorescence for distinguishing *Rubia akane* red (Japanese madder) from other red dyes in Shosoin textiles over 1250 years old. The characteristic peak at λ_{ex} 371 nm in references confirmed the presence of *R. akane* red in the historical samples(Fig. 4). However, there was a weaker emission peak for the historical samples compared to the reference because of smaller quantities of the dye components. Claro et al. [76] employed micro-spectrofluorimetry to record emission and excitation spectra with high spatial resolution (8–30 µm) and identified purpurin and pseudopurpurin in Paracas and Nasca textiles dated from 200 B.C. to A.D.1476, and also carminic acid in textiles dated close to the Inca Empire of A.D. 1000–1476. Results from microspectrofluorimetry agreed with those obtained requiring micro-sampling, such as HPLC-DAD-MS.

Reflectance spectroscopy is a well-established technique for the characterization of paints and pigments [77], however, its application to textile dyes is rather limited. Qualitative reflectance spectral data could usefully indicate the presence or even identity of dyes when sampling and extraction are not possible, thereby offering a useful preliminary tool prior to judicious micro-sampling for invasive analytical procedures [78]. The possibility to examine a large set of points on the textile for a first interpretation of results in a short time enables greater precision and significance to choose areas for invasive examination [79]. Despite its high degree of selectivity and popularity in art diagnostics, reflectance spectroscopy has not yet become a popular analytical tool for organic colorants on textiles. This is because of poorer wavelength resolution and 'fingerprinting', combined with difficulties to couple the analyzed surface efficiently to the instrument [77]. However, with development of fiber optics reflectance spectroscopy (FORS), an outcome of the application of fiber optics technology to this technique, reflectance spectroscopy has emerged as a powerful surface analytical technique that is able to produce reflectance spectra for materials such as dyes and pigments [80, 81]. Although several variables and ambient factors might adversely affect the acquisition of reliable spectra, FORS in the UV, visible and IR regions is considered simple and cost effective methods for *in situ* characterization of dyes on ancient textiles [82]. The material under investigation is illuminated through a fiber optic probe and the reflected light is captured and measured to produce a characteristic reflectance spectrum for the material. Dye identification is achieved by comparing the spectral features (inflection points and reflection minima, i.e. maxima in apparent absorbance coordinates) of the investigated unknown spectrum with those for known materials. Anthraquinone dyes can be identified by an absorption band between 500 and 570 nm structured into two sub-bands. It is possible to distinguish dyes of animal and vegetal origin: sub-bands occur at 520/540 and 560/580 for the former; and at 500/515 and 535/540 for the later [72]. However, more detail is usually

harder, for example, the different scale insect dyes have very similar spectra, andreliable spectral responses from the surface strongly depends on the preservation condition of the sample. FORS has already proved effective for identification of anthraquinone dyes in historical textiles [19, 72, 73, 82, 83]. Angelini et al. [82] successfully identified cochineal as source of red colorant in a Yörük rug by comparing FORS spectrum of a red areawith the reference spectra of cochineal and lac. The positions of the absorption bands in historical and reference samples superimpose perfectly, but the maxima of the reflectance spectra in the blue region as well as the shape of the curves in the range 600–700 nm suggest the presence of cochineal rather than lac. In another investigation, Gulmini et al. [72] found that Vis-FORS can readily discriminates between animal and vegetable red dyestuffs, but direct identification of the dyestuff via Vis-FORS greatly depends on the concentration of the dyes on the fibres, since colours that are too intense or too faint produce spectra where the characteristic bands cannot be observed. Another successful example is non-invasive analysis with a combination of FORS and FLD to detect scale insect red and distinguish textiles dyed with anthraquinone dyes combined with woad/indigo from true molluscan purple in Coptic textiles from the Museo Egizio in Torino, Italy [83]. Absorbance is an additive property, so the occurrence of double dyeing can be detected providing that the absorption bands of the two species are resolvable; this is typically the case with textiles dyed in sequence with woad/indigo and anthraquinone dyes [83]. Very recently, Maynez-Rojas et al. [19], successfully identified cochineal and brazilwood for red and a mixture of cochineal and indigo for purple in 19th and 20th-c. textiles by comparing FORS spectra of historical samples with unaged and aged reference samples prepared in laboratory conditions (Fig. 5). The researchers highlighted that a comprehensive database of FORS spectra, including dyes, supports, dyeing recipes and aging periods, is essential for the identification of the dyes present on cultural heritage objects.

3.2 Surface enhanced Raman spectroscopy (SERS)

Conventional Raman spectroscopy, while extremely successful for the identification of inorganic pigments [84-86], is not suitable for the identification of several important organic dyes because the intense fluorescence of many organic colorants hinders the measurement of Raman scattering [87, 88]. This can be overcome with near-IR excitation lasers at 1064 nm which yield good enough responses as a consequence of significant reduction in fluorescence [89]. In recent years, SERS has emerged as a powerful technique for micro-destructive or minimally invasive analysis of historical organic colorants, allowing highly sensitive structural detection, low sample requirement, dramatic fluorescence quenching and significant enhancement in Raman signals of target analytes [8, 21, 90-93]. Several groups have demonstrated the suitability of SERS application in historical dye analysis of artwork, textiles and other cultural heritage material, as presented in several recent reviews [8, 18, 21, 92-94]. The main advantage of SERS lies in its minimal sampling requirements and high sensitivity, making it possible to identify target molecules on fibre microsamples measuring 20 µm to 100 µm. Despite significantly-reduced sample size and analysis time, SERS is limited in historical dye identification by three main factors: (i) necessary preparation of various colloids and considerations related to plasmon conditions to enhance the Raman scattering effect, (ii) inability to resolve dye mixtures because SERS spectra are the aggregate response of all components, and (iii) tendency towards preferential detection of components with an affinity for the SERS substrate, resonance and solubility [93, 95, 96]. When such limitations can be overcome, SERS offers an ideal technique for anthraquinone dye identification for various reasons, these being that their extended molecular π-electron systems or presence of atoms carrying lone pairs of electrons adsorb well onto metal nanoparticles; their conventional Raman spectra are obscured by very high backgrounds due to extreme fluorescence; and they are detected in very low quantities in historical samples because of their high tinctorial power

[88]. The first application of SERS for the identification of dyes of historical interest was demonstrated by Guineauand Guichardin [97] in 1987 when they obtained spectra for alizarin extracted from madder-dyed textiles using Ag electrodes as the SERS-active substrate [88]. Several SERS substrates have been tested to improve the quality of enhanced Raman scattering and reproducible spectra to achieve conclusive identification of anthraquinone dyes, and the successful common substrates were Ag colloids [88, 91, 92, 98-104]. The preparation of silver colloids for textile dye analysis usually involves chemical reduction of silver nitrate with sodium citrate by the method proposed by Lee and Meisel [88, 91, 92, 99-104]. Alternative approaches for the preparation of silver colloids entail reduction with hydroxylamine and microwave-supported glucose reduction of silver sulphate in the presence of sodium citrate as a capping agent [105]. Usually a drop of silver colloid is deposited on the sample and SERS spectra are collected before its complete evaporation or after drying [88, 103, 105]. Jurasekova et al. [106] proposed a method to produce Ag nanoparticles *in situ* via laser photoreduction of silver nitrate aqueous solution directly in contact with the fibre.

One of the main problems encountered with the Ag colloid approach relates to the difficulty of obtaining stable adsorption of anthraquinones on the surface of solution-reduced colloids. Particles prepared by reduction from solution with sodium citrate as a reducing agent are surrounded by a layer of negatively-charged citrate ions, which impedes the stable absorption on the nanoparticles of acidic or phenolic species such as anthraquinones [106]. To overcome this problem, alternative approaches using other SERS substrates have also been proposed: electrodes [97], Tollens mirrors [88], Ag nano-islands and films [88, 107, 108], silver film over SiO_2 nanosphere [104], Ag-agar gel [109, 110], and Zr-Ormosil polymer [111]. In a comparative investigation into the suitability of SERS substrates, Leona et al. [88] concluded that citrate-reduced Ag colloids provide superior sensitivity for anthraquinone dye detection compared to hydroxylamine-reduced Ag colloids, Tollens mirrors and silver

nano-island films. One of the main limitations of SERS has been that, while it has proven greatly successful with pure dye compounds and model reference materials, results on actual historical samples are not equally favourable. This is because, apart from target analytes, the significant enhancement in Raman signals can involve other species present in complex samples if they have affinity for SERS substrates. Another common problem is the appearance of anomalous bands that arise sometimes in the SERS spectra on colloids. Use of poly-L-lysine and ascorbic acid as aggregating agents, with addition first of the analyte and subsequently of suitable electrolytes to the colloid in an inverted order, could be helpful in limiting the intensity of such spurious bands [101]. Much of the works conducted in the SERS for anthraquinone dye analysis focused on the characterization of reference substances such as alizarin [88, 98, 100, 107, 111], purpurin [100, 107], carminic acid [88, 107], kermesic acid [88], laccaic acids [99, 112], and historical anthraquinone lake pigments [94, 107, 95] for interpretation of their spectral patterns, with a few reports also investigating reference dyed samples [106-108] and actual historical textiles (Table 2) [101, 104-106, 110, 113].

SERS investigations on historical textiles have demonstrated the possibility of detecting anthraquinone dyes in few hundred micrometer-length samples [94]. Distinctive SERS spectra of even closely-related dyes makes it possible to identify characteristic compounds through spectral matching with references, although manually matching spectra from unknowns often turns out to be cumbersome and not always effective [93]. The availability of extensive spectral databases as well as accessible library search methods designed to match a query spectrum to the closest library references are crucial to ensure faster and more reliable positive identification [93, 114]. SERS analysis for dye analysis in textiles includes both extractive and non-extractive approaches [88, 103-105, 113, 115]. To avoid the problematic issues of dye extraction and sample size, recent works employed on-the-fibre technique for SERS on textile microsamples [92]. On-the-fibre extractionless SERS offers an excellent tool to

characterize historical dyes with minimal preparation, especially when sample extraction would not be possible because of sampling restrictions or risk of dye degradation during extraction [104], however its application to cultural heritage applications is limited because the preparation and application of Ag colloids is a chemical interacts with the tested material, therefore it can alter the object while posing the possibility of future unknown sensitisation or damage. By highlighting the SERS incompatibility of techniques traditionally used to extract dyes from historical samples, Leona et al. [88] proposed a milder alternative non-extractive hydrolysis approach. A very small fragment of textile fibre is removed from the object and treated with HF vapours in a microchamber to detach the dye from the mordant. A droplet of citrate-reduced Ag colloid was then added to the sample to deposit free dye molecules on the surface of colloids. The authors successfully demonstrated its applicability by identifying alizarin in a microscopic sample of a single fibre from a red thread in a 16th century tapestry. A similar methodology was used to characterise anthraquinone colorants in textiles from different sources [92, 102, 103, 114], and has been demonstrated to have the potential to distinguish closely related anthraquinones [103].

Another method gaining popularity is extractionless non-hydrolysis approach, where Ag colloids are applied on the fibre microsamples without HF treatment [91, 104-106]. Brosseau et al. [104] used this for the first time *in situ* "on the fiber" SERS to identify lac and cochineal dyes in red and pink fibers from historical textiles, demonstrating the utility of extractionless non-hydrolysis SERS for detection of anthraquinone dyes (alizarin, purpurin, carminic acid and lac dye) using citrate-reduced Ag colloids for dyed reference fibers and actual historical textile samples. Jurasekova et al. [106] used silver nanoparticles fabricated by laser photoreduction for successful on-the-fiber detection of alizarin in Coptic textile of Egyptian origin. In another study, Idone et al. [91] demonstrated direct, extractionless, non-hydrolysis SERS identification of two dyes simultaneously on a historical sample by

consistently detecting cochineal and brazilwood in both the silk velvets and cotton fibers. The spectra of reference dyed samples collected on the same sample were reproducible in terms of position of the peaks, however the relative intensity of the signals varied, possibly due to the proximity of the dye near the SERS substrate or a varying concentration of dye and Ag colloidal paste at different locations on the fiber. The authors cautioned that lack of SERS signal in very light colored historical samples is a consequence of a very low concentration of dye, and in such cases extractive methods could be more useful. In an investigation on sample treatment considerations in the analysis of organic colorants by SERS, Pozzi et al. [105] observed that spectra obtained without hydrolysis generally display lower intensity and poorer signal-to-noise ratios compared to those taken after HF treatment. However, the non-hydrolysis SERS approach was found to be more appropriate for dyed silk because HF-treated samples yielded very poor spectra probably due to the interference of proteins released into solution following hydrolysis. Considering the relative merits of both non-extractive and extractive procedures and their mutual compatibility, the authors recommended a two-step procedure for analysis of unknown samples: analysis of the sample first without the hydrolysis step, and then removing the colloid and exposing the same sample to HF.

Another concern for SERS analysis is the requirement of microsamples removal from the objects. To further minimize the damage incurred to irreplaceable objects, recent efforts have been directed to developing minimally-invasive methods that can deliver the SERS-active nanoprobe to the object without the need of sampling [93]. A promising example is Ag-embedded polymer gel loaded with a mild micro-extractant, which can be directly applied to the area of interest on the artefact [109]. Ag-agar gel is prepared by mixing the polymer gel with Ag colloid to make a gel bead loaded with a solvent or chelating agent, which is placed on the area of interest to extract a minute quantity of dye from the confined area on the object

in contact with the gel [110]. After removal of the gel from the sample, SERS spectra are recorded by focusing the laser beam directly onto the gel surface. An Ag-embedded gel matrix is an excellent absorbent probe for the micro-extraction of dye molecules from textiles and efficient enhancer of Raman scattering due to the silver nanoparticles trapped in its structure. Ag-agar gel has been successfully tested as a SERS substrate on mock-up textiles to identify alizarin, purpurin and carminic acid and on a pre-Columbian textile, revealing the presence of alizarin (Fig. 6) [109, 110].

A proof-of-concept study described the possibility of identifying anthraquinone dyes on textiles without extraction sampling by a ‘‘dry-state’’ SERS approach, i.e., by exploiting the interactions between a solid nanometallic substrate and dye molecules present on textiles [108]. SERS-active silver island films were used to obtain SERS spectra of reference dyes in solution and dyed wool samples by the ‘‘dry-state’’ approach, and results were compared with conventional Raman analyses. Dyes exhibited high fluorescent background in conventional Raman spectroscopy, while measurements using ‘‘dry-state’’ SERS, directly from dyed wool samples, produced well recognizable SERS spectra of alizarin, purpurin and lac dye in good accordance with SERS spectra of solutions of the same dyes deposited on silver nano-islands. Since the sample areas under investigation are very small (ca. 10 μm), the dimensions of the support on which the metallic substrate is deposited could be reduced to the diameter of a fibre optic distal tip, opening a new window of opportunity toward non-destructive SERS analysis of historical objects.

A systematic study to determine the SERS technique’s ability to discriminate closely related molecules in binary mixtures by Pozzi et al. [96] used a selection of natural reds including alizarin, purpurin, carminic and laccaic acids. Rambaldi et al. [103] demonstrated its utility in differentiating among closely related anthraquinone derivatives and suitability for detecting pseudopurpurin in reference dyed wool samples. In another study, Pozzi et al. [114]

introduced statistical methods and library search approaches for fast and reliable identification of dyes using SERS. By coupling SERS with principal component analysis (PCA) and a spectral search program based on the correlation coefficient (CC) algorithm, they successfully assigned the reference spectra to dye groups and classified 20 SERS spectra of unknown colorants from works of art and museum objects by searching them against the library. By applying this approach, they successfully identified many madder, cochineal and lac anthraquinones in selected historical textiles by reliably matching against a well-constructed spectral library.

The recently proposed coupling of SERS with TLC [104] and HPLC [116] has offered alternative ways to resolve and identify individual components in dye mixtures. Continuous efforts are being made to improve SERS detection of historical dyes and the future of this powerful molecular spectroscopic technique in this area of application looks promising.

3.3 Techniques based on Direct Mass Spectrometry

In the last few years, mass spectrometric techniques such as direct analysis in real time – time-of-flight mass spectrometry (DART-TOF-MS) [16, 17, 117], laser desorption ionization (LDI)-MS [118], matrix assisted laser desorption ionization (MALDI) [118, 119], time-of-flight-secondary ion mass spectrometry (TOF-SIMS) [120] and liquid microjunction surface sampling probe mass spectrometry (LMJ-SSP MS) [121] have become popular as alternative approaches in the analysis of organic dyes in textiles. These direct methods can provide information about the dye from samples as small as fibres, and avoid the disadvantages of sample preparation by extraction, hydrolysis, or derivatization [121], as shown by a rapid, simple method for the identification of anthraquinone dyes in textiles by DART-TOF-MS [16, 17, 117, 122]. Small fibre samples taken from the objects were introduced directly with forceps into the gap between the DART source and the mass spectrometer orifice where ionisation is carried out directly on the sample, and exact mass determinations made within

minutes. A DART-TOF-MS analysis of fibres from a 19th century treatise produced strong alizarin signals at m/z 241.050 Da within a few seconds of exposure to the DART gas stream [17]. In many dyed textile samples, an unidentified peak at 257.24 Da can be easily distinguished from purpurin (257.045 Da) because TOF-MS has high mass resolution. Two minor anthraquinone components of madder -pseudopurpurin at m/z 301.035 Da and rubiadin at m/z 255.067 Da, with mass differences of 0.36 and 0.86 mDa respectively for the M + H^+- were also identified. Several anthraquinones characteristic of dyes from *Gallium* spp. genus were identified in textiles from prehistoric Eastern North America by DART-TOF-MS in negative ion mode, which was more sensitive than positive ion mode [117]. Armitage et al. [16] investigated the *Relbunium* and cochineal colorants in Paracas Necropolis textiles by DART-TOF-MS and found that *Relbunium* anthraquinones were readily distinguished while the aglycone carminic acid from cochineal was less easy to identify with certainty. DART spectra of references of anthraquinone compounds predominant in *Relbunium* species showed that while compounds of the same molecular formula yield identical spectra for the parent ion at −30V (no fragmentation), the fragmentation patterns at −90V (fragmentation due to collision induced dissociation) can be used to differentiate structural isomers. DART-MS analysis of Paracas textiles indicated purpurin and xanthopurpurin as the primary anthraquinones, as expected for dyes from *Relbunium* plant roots, with munjistin and pseudopurpurin also identified in some samples. Napolitano et al. [118] tested LDI-MS/MS for *in situ* detection of alizarin in a swatch of dyed silk. The full-scan spectrum of the sample showed few relatively abundant ions: m/z 223, 501, 550. Peaks for alizarin in its unreduced (P) and reduced (P+1) forms, at m/z 241 and 242 respectively, were not prominent in the normal mass spectra, therefore MS/MS was performed on both. Comparison of neutral losses in daughter ion spectra of P and P+ 1 with the standard confirmed *in situ* detection of reduced alizarin in the dyed textile (Fig. 7).

Very recently, Kramell et al. [121] proposed a rapid and minimally-invasive dyestuff analysis under ambient conditions through real-time *in situ* microextractions of fibres by flowprobe™-ESIHRMS (high-resolution MS), a commercial LMJ-SSP device coupled to an Orbitrap mass analyzer. The fibres were simply fixed on a conventional glass slide by wetting with a drop of solvent and characteristic dyestuff signals were detected within a few seconds regardless of the fibre types, age of the sample, or the sample's appearance. The system used a pair of coaxial capillaries positioned close to the surface of the object where a continuous solvent flow forms a liquid micro-junction between the probe and the sample surface and delivers extractable analytes to the ESI mass spectrometer. The method successfully confirmed madder and cochineal in five red-shaded samples from the collection of the Ethnologisches Museum—Staatliche Museenzu Berlin. Three samples showed spectra similar to madder-dyed reference fibres, with $[M–H]^-$ signals for dihydroxyanthraquinones (alizarin and xanthopurpurin, m/z 239.0347), trihydroxyanthraquinone (purpurin, m/z 255.0298), dihydroxy-methyl-anthraquinones (rubiadin, m/z 253.0505) and dihydroxyanthraquinone-carboxylic acid (an unknown isomer of munjistin, m/z 283.0249). One sample was found to be dyed solely with cochineal, having HRMS spectra with signals for carminic acid and its isomers (m/z 491.0834), flavokermesic acid (m/z 313.0035), kermesic acid (m/z 329.0357) and a dcII-like compound (m/z 475.0881).To distinguish between the isomeric structures, HPLC-MS/MS and HPLC-DAD were used to compare their spectra and retention times with authentic reference compounds. In another sample, signals for cochineal anthraquinones and rubiadin and purpurin were detected, suggesting cochineal witha plantred dye sources like *Relbunium* spp. Although LMJ-MS is limited for fragmentation pattern interpretation to identify and assign isomeric structures, this can be overcome by incorporating an LC system between the surface sampling device and the mass spectrometer.

3.4 High Performance Liquid Chromatography (HPLC)

Wouters[13] introduced HPLC with UV-Vis detection for separation and identification of dyes in historical textiles while working on anthraquinone dye characterization from Coptic textiles dating from 3rd to 8th centuries. A number of papers have been published in the last three decades underpinning the robustness and versatility of HPLC-based methods to characterize organic colorants from a variety of historical textile artefacts [7, 15, 22]. HPLC is now an established routine method for dye analysis, where the dye sources are usually identified by comparing the chromatographic profiles of extracts of historical samples with reference materials[14, 15]. HPLC systems equipped with increasingly improved detectors, such as DAD, MS, FLD and their combinations, have proved successful for identifying anthraquinone components and their sources in a variety of historical and archaeological textile objects (Table 3). DAD seems to be the most efficient and widely used detector for both quantitation and confirmation of the identity of organic dyes in complex textile matrices by spectral matching with reference materials, while MS detection offers solutions to problems encountered in identification of unknown dye components, especially when reference substances are not available [7, 14, 15]. Moreover, MS studies can provide useful insights on the nature of degradation products when ageing studies are performed.

3.4.1 Sampling and dye extraction protocols

Sample preparation is a crucial step before chromatographic and spectroscopic analyses, which shapes the success of all downstream analytical processes [23]. Informed selection of sample preparation methods and extraction protocols are of immense importance in HPLC analysis of historical samples to maximise chances of successful analysis to justify the removal of fibres from precious cultural heritage artefacts [123]. The quality and quantity of isolated colorants depend greatly on the extraction procedure employed. Usually, a very small amount (0.5–1 mg) of yarn or thread of a single color is taken from the textile object in

question; dye components are extracted for further analysis [7]. A typical sample preparation procedure involves dye extraction from the fibre with appropriate solvent followed by solvent evaporation and re-solubilisation of dried residue [7]. A number of different approaches have been successfully employed for extraction of anthraquinone colorants from historical textiles, which could be broadly grouped into three distinct categories: (i) extraction with acids, (ii) extraction with organic solvents and (iii) extraction with complexing agents. An extensive list of dye extraction methods from historical textiles for HPLC analysis is presented by Pauk et al. [14].

The earliest sample preparation procedure for HPLC analysis of anthraquinone dyes involved heating of dyed sample in water/MeOH/37% HCl (1:1:2, v/v/v) for 10 minutes at 100°C, followed by drying over sodium hydroxide in a desiccator, and resolubilising the residue in water/MeOH (1:1, v/v) just before analysis [13, 33, 45, 124]. Natural anthraquinones were mainly used as mordant dyes, where the carbonyl and adjacent phenol groups participate in the fibre–mordant–dye complex [1]. Since the strong action of HCl disrupts the dye–metal complex, allowing the extraction of the anthraquinone colouring matter into an aqueous/organic solvent, hydrolysis with strong acids at elevated temperatures has been the most widely-suggested and applied dye extraction procedure. However, HCl-based extraction sometimes causes unwanted excessive hydrolysis, decarboxylation and glycosidic bonds cleavage in chromophores leading to loss of valuable information, which may be critical in identifying a specific source from which the dye was obtained [41]. Partial hydrolysis of pseudopurpurin and munjistin was reported by Wouters [13] during dye extraction from textile fibres in the original HCl-extraction procedure. The HCl-based extraction procedure is still a widely accepted approach [41, 54, 125-132] with modifications to hydrolysis conditions [116, 133, 134] and use of alternative solvents solvents (MeOH [116, 135], DMF [136], MeOH/DMF [137, 138], MeOH/formic acid [139], DMSO [50, 140, 141]) to solubilise

the resulting dried residue. The treatment of non-dissolved residue with pyridine [70, 133, 134, 142], an additional first-stage extraction step using MeOH/DMF [137], or DMSO [143] and sonication [144, 145], have all been suggested for enhanced dye recovery too. Although these improved HCl methods provide good yields of extracted dye, hydrolysis of glycoside-containing colorants into their aglycones, low yield of acid-sensitive anthraquinones and the destruction of the fibre result in HPLC profiles cannot be conclusively matched to the corresponding anthraquinone sources [38]. High background in the UV range is also observed, due to the presence of some hydrolysis products of the yarns. To avoid these inherent limitations of the harsh HCl-extraction method, several alternative extraction procedures were developed and compared by various researchers. These 'soft' or 'mild' extraction procedures, which allow for a better recovery of easily degradable components and labile dye chromophores, involve use of EDTA (ethylenediaminetetraacetic acid) [42, 146-148], TFA (trifluoroacetic acid) [146, 149, 150], formic acid [10, 146, 151, 152], oxalic acid [32, 146], citric acid [41, 146], DMSO [153] and glucose [41]. There are several reports on comparing the extraction efficiency of dye recovery procedures employed in HPLC analysis of historical textile dyes. Considering the problem of glycoside decomposition, Zhang and Laursen [148] developed two mild extraction protocols: the first with formic acid: MeOH (5:95, v/v) at 40 °C for 30 min and the second used H_2EDTA: acetonitrile: MeOH (2:10:88, v/v/v) at 60 °C for 30 min. While both methods gave higher extraction yields than the traditional HCl-method, formic acid method was more suitable for anthraquinones extracted from textiles dyed with madder and cochineal. Zhang et al. [10] later employed the formic acid method to successfully extract and identify the significant anthraquinone glycosidic components ruberythric acid and lucidin primeveroside as well as the major aglycones components alizarin, purpurin, rubiadin, munjistin and xanthopurpurin in red threads from ancient Chinese textiles.

Valianou et al. [146] compared the efficiencies of five extraction methods (HCl, citric acid, oxalic acid, TFA and a combination of formic acid and EDTA) for (a) number, (b) relative quantities and (c) signal-to-noise ratios (S/N) of the compounds extracted from reference dyed wool sample. All the tested methods were successful in extracting carminic acid, with the oxalic acid method as the most efficient. For alizarin and purpurin, HClextraction was found to be most efficient, and formic acid–EDTA and TFA methods also effective. A complete evaluation of an extraction method should include S/N measurements to determine background noise in the chromatogram from the substrate. The S/N for the HCl method results was significantly suppressed compared to the TFA and oxalic acid methods (Fig. 8), important for increasing the limit of detection (LOD). Subsequently, Valianou et al. employed the TFA method on Mamluk period silk textiles and successfully identified alizarin, purpurin, lucidin, carminic acid, kermesic acid, laccaic acid A, flavokermesic acid, dcIV and dcVII in these historical samples. In another comparative evaluation of dye extraction method from wool samples Manhita et al. [138] highlighted the mildness and efficiency of a Na_2EDTA/DMF compared to other extraction methods. Serrano et al. [51] tested four extraction protocols for cochineal dyed reference textiles: (a) formic acid-MeOH (5:95, v/v), (b) HCl 37%- MeOH-H_2O (2:1:1, v/v/v), (c) oxalic acid (0.2 M)-acetone:MeOH-H_2O (0.1: 3: 3: 4, v/v/v/v) and (d) TFA 2 M. The oxalic acid and TFA methods yielded superior extraction efficiency compared to others; however, the TFA method showed better chromatographic resolution and a higher level of agreement between PCA (principal components analysis) scores from the extracts from cochineal-dyed reference textiles and those from reference *D. coccus* insect specimens. The mild character of an alkaline mild extraction technique involving ammonia with disodium EDTA (ammonia-Na_2EDTA method) has allowed the identification of different anthraquinone glycosides in dyed yarns and lake pigment specimens prepared with madder [42], American cochineal and Armenian cochineal

[147]. Very recently, Ford et al. [41] proposed a new aqueous glucose solution extraction and compared it with three other extraction methods (HCl, oxalic acid and TFA). The glucose solution acts as a favourable extraction medium due to its ability to form extensive hydrogen bonding with madder glycosides, and thus can displace them from the fibre. The method was successful in extracting acid-sensitive molecules of madder such as glycosides (lucidin primeveroside and ruberythric acid) and sensitive aglycones (lucidin) both in reference dyed and artificially-aged wool. This new glucose method enhances the possibility of detecting acid sensitive glycosides contained in the dyed textiles, and thus can provide further information about historical dye preparation and dyeing processes with respect to current methods.

Although these soft methods of extraction have been proposed as alternatives to the harsher acidic extraction, they typically result in less effective color removal from the substrate. Surowiec et al. [137] proposed a method including MeOH/DMF extraction as an additional step in the acidified MeOH extraction process for enhanced recovery of extracted dye components. Resulting chromatograms of extracts from red/brown thread of the Dava Moor collection showed higher peaks for alizarin and purpurin for extraction with additional step (Fig. 9). A two-step procedure involving mild hydrolysis followed by the traditional hydrochloric acid method has also been proposed as a better alternative to a one-step procedure [154, 155]. Mild extraction conditions in the first step allow isolation of glycosides and acid-sensitive anthraquinones, while the second step where the harsh conditions are applied leads to recovery of aglycones [141, 151]. The identification of these sensitive and rarely-detected components could provide a complete view of the molecular pattern involved in dyeing processes, and thus could help in differentiating species of *Rubiaceae* and other anthraquinone sources [42, 147].

3.4.2 Chromatographic separation

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most common mode for separation of historical anthraquinone dyes [15]. The most employed experimental procedure for the RP-HPLC analysis of anthraquinone dyes generally involves a C18 [13, 42, 131, 144, 146, 150, 156], C8 [143] or C4 [10, 148] based non-polar stationary phase and mobile phases consisting of pure water (aqueous) and acetonitrile or MeOH (organic) eluents [15], with formic acid [6, 148, 157, 158], trifluoroacetic acid [131, 136, 146, 150, 159], phosphoric acid [137, 160] or methanesulphonic acid [161] added as mobile phase modifiers. The most commonly employed range of stationary phase particle diameters for RP-HPLC is 3–5 µm. A preliminary investigation by Surowiec et al. [137] on comparative detection limits from the 4.6 mm internal diameter (i.d.) column with a narrow-bore C18 column (2.1 mm i.d.) indicated an increase in the detector response for the narrow-bore column, providing enhanced sensitivity. Gradient elution, where the composition of the mobile phase changes over a period (in terms of mobile phase modifier or the solvent composition), is generally preferred over isocratic elution because it allows greater flexibility in the separation of dye components. Selection of an appropriate gradient elution method is a critical factor for acceptable elution and resolution of structurally related anthraquinones.

The introduction of UHPLC, which uses columns packed with sub-2 µm porous particles and an instrumental setup enabling extreme pressures, has further facilitated the detection of analytes in sub-picogram ranges along with dramatic improvements in resolution, sensitivity, and speed of analysis [162, 163]. Serrano et al. [164] compared the separation of flavonoids and anthraquinones on a UHPLC system employing a Waters BEH Shield RP18 column (1.7 µm particles) with a HPLC system using a Phenomenex Luna C18 column (3 µm particles) (Fig. 10). In most cases UHPLC provided chromatograms with better resolution, lower limits of detection and lower limits of quantification. Significantly improved limits of detection

with UHPLC allow the acquisition of more precise data which is extremely valuable in characterization of minor dye components [165].

3.4.3 Detection of separated dye compounds

3.4.3.1 Absorbance detection - UV, Vis, and DAD Detectors

Absorbance detection finds extensive usage in the identification of anthraquinone dyes because these compounds respond well to the UV-Vis region of the electromagnetic radiation spectrum (Table 4) [166]. The first application of HPLC with UV-Vis detection to anthraquinone dyes of historical interest was reported in 1985. The data acquisition and interpretation was carried out only at 255 nm for successful identification of a mixture of plant (alizarin, purpurin, pseudopurpurin and munjistin) and insect (carminic acid and kermesic acid) anthraquinone dyes in a 13th-century tunic of a Spanish bishop, fibres from a 15th-century tapestry, and Coptic textiles [13]. HPLC analysis of dyed yarns from different historical sources indicated that the comparison between the relative retention times of significant peaks and their UV-Vis spectrum can identify the most probable plant/insect source(s) of the dye [13, 33]. Most papers on HPLC analysis of anthraquinone dyes report the use of UV-Vis detection, and with the introduction of DAD, also known as photodiode array detectors (PDA), more reliable identification of dyes can be done [13, 14, 126, 136, 167-170]. The simultaneous detection at multiple wavelengths is especially important for dyed historical samples because components from dye sources and degradation besides anthraquinones can be present which absorb at different wavelengths. Today, HPLC-DAD has become a routine strategy for the detection of anthraquinone dyes in historical and archaeological textiles. Some of the most recent examples of identification of anthraquinone dyes in archaeological textiles based on HPLC-DAD setups are the detection of laccaic acids, alizarin and purpurin in 1st century AD textiles from 22nd Noin-Ula barrow, Mongolia (Fig.

11a) [139] and an assessment of the presence of different madder (*R. tinctorum*L.) anthraquinones with indigotin in late 2nd millennium BCE textile fragments from excavations in Timna Valley, Israel (Fig. 11b) [153].

While HPLC-DAD is the most successful technique to date for anthraquinone dyes identification in historical textiles (Table 3), it sometimes lacks selectivity for precise detection leading to uncertainties in the identification of the dye components. The UV–Vis absorption of chemical components is influenced by the mobile phase composition, so difficulties can arise in differentiating components with similar UV–Vis absorption due to the lack of fine spectral details [141]. Another issue in the analysis of historical dyes is the presence of secondary degradation products and artefacts of sample treatment in the extracted dye mixtures, generating unknowns in HPLC chromatograms [14].

3.4.3.2 Fluorescence detection

Fluorescence detection is useful only for compounds characterised by a significant fluorescence quantum yield. Fluorescence detection has found very little applicability in historical dye analysis due to the limited number of strongly fluorescing dye compounds found in historical objects. This inherent disadvantage makes fluorescence detection very selective towards specific analytes. On the other hand, the limited fluorescence of several analytes can be overcome by converting them into fluorescent derivatives bypre- or post-column derivatization [22, 134, 175]. The high sensitivity of fluorescence detectors makes them particularly useful for trace analyses in the field of heritage science because the amount of samples is generally limited and the concentration of organic dyes often extremely low in a historical textile matrix [171]. Eluted solutes can be detected down to the low picogram level at fixed wavelengths of excitation and emission in a fluorimeter [172]. Fluorimetric detection is especially useful for providing complementary information to characterize dye compounds

with a weak UV absorption when absorbance detectors cannot identify them conclusively. Some anthraquinone dyes such as carminic acid [173] show natural fluorescence while others show an increase in fluorescence signal after complexation with metallic ions [134]. Post-column complexation of anthraquinone dyes with gallium ions has been used in their determination in extracts from Coptic textiles [134, 174] and with zirconyl ions in extracts from 19th century tapestry from Aubusson (Table 5) [175].

3.4.3.3 Mass spectrometry detection

HPLC systems equipped with MS detectors provide high discriminating power for the identification of historical dyes that cannot be reliably distinguished based on their UV-Vis absorption profile. The molecular mass of separated analytes and structural information from the fragmentation pattern under certain instrumental conditions or a particular mass spectrometer can identify the dye compound [7]. Moreover, MS detection (and in particular, tandem MS detection) offers better LODs, improved selectivity, and the possibility for structural elucidation of unknown dye components [14, 143]. The use of high-resolution mass spectrometers can also provide the raw formula of unknown species, aiding their characterisation. Occasionally, MS detectors are employed in combination with DAD detectors (HPLC-DAD-MS) as a complementary tool for additional information to identify analytes unambiguously [148] by providing simultaneous analysis of their retention times, UV–Vis absorption spectra and molecular characteristics. A full scan MS with different ionization modes combined with simultaneous UV detection is often sufficient to detect the main dye compounds by plotting traces for molecular ions of investigated compounds besides retention time matching [134]. Additionally, in tandem mass spectrometers, the analysis of the data from the full scan mode can provide a list of precursor ions to be subjected to further structure elucidation by collision induced dissociation (CID), followed by the detection of the product ion formed [127, 134] . Yamaoka et al. [176] employed LC–MS with thermospray

ionization and selected ion monitoring to investigate red dyes in woven fabrics from the Greco–Roman period allowing detection and identification of alizarin. The advent of soft ionization techniques, i.e., electrospray ionisation (ESI) and atmospheric pressure chemical ionization (APCI), has further boosted interest in MS detection of anthraquinone dyes [7]. ESI is the most important soft ionization technique in the analysis of anthraquinone dyes due to their polar nature [32, 51, 141, 147, 151, 177-181], though APCI has also been used in few reports [50, 127, 177]. In particular, ESI in an LC-tandem MS configuration that is widely used in detection and structural characterization of anthraquinone dyes from reference compounds, dyed samples and historical textiles [145, 152, 178-180]. Anthraquinone detection has been reported in both positive and negative ion modes, however negative ion mode is reported to be more sensitive and typically preferred [54, 151]. HPLC with time-of-flight mass spectrometry has also been successfully employed to detect alizarin and purpurin in a Tang dynasty textile [182].

Investigations of Coptic textiles from the Early Christian Art Collection at the National Museum in Warsaw showed that the detection capabilities of the LC-MS system utilizing different scanning modes of a triple quadrupole mass spectrometer were comparable with UV-Vis and fluorescence detection [133, 134]. Szostek et al. [133] identified alizarin and purpurin in the extracts of Coptic fibre samples based on retention time, UV-Vis and tandem mass spectra of reference materials (Fig.12). Kohout et al. identified madder alone or in combination with one, two or three other sources of dyes in archaeological textile fragments dating to the 14th and 15th centuries by using LC-HRMS (High resolution mass spectrometry) analyses [183]. Surowiec et al. [143] carried out an optimization of LC-MS detection of natural colorants including anthraquinones in three acquisition modes of MS analysis: scanning, selected-ion monitoring (SIM), and multiple reaction monitoring (MRM) in both positive and negative ion modes. Although they reported better LODs for MS

detection, measurements with the DAD were more repeatable. Rafaelly et al. [184] optimized conditions for HPLC-ESI-MS detection of anthraquinone dyes by adjusting source parameter step-adjustment, mobile phase composition and post-column additive testing, and successfully applied it to the identification of components in reference wool samples dyed with *Rubia tinctorum* and *Galium verum*. In both [133] and [184], the data on the fragmentation in CID of the most important anthraquinones are reported. The fragmentation scheme is also provided and discussed in detail in [133] and shown in Fig. 13.

As major colouring components are often the same in closely related dye sources, the characterisation of the whole chromatographic profile, including the identification of minor constituents, is fundamental to assess the particular sources of natural dye in historical textiles. In some cases, relative amounts of minor components can be used to assess the origin of a dyeing material. The exact structures of the dcII, dcIII, dcIV, and dcVII components of cochineal found in historical textiles has been subject of debate for a long time [50]. MS detection in both negative ESI [50, 166] and negative ACPI [50, 177] modes have shown that the deprotonated molecular ion of dcII and dcIII corresponds to m/z 475 and 490, respectively, and the deprotonated molecular ions of dcIV and dcVII to m/z 491. These compounds formerly described as dcII and dcIII, were identified as 2-C-glucoside of flavokermesic acid and 4-aminocarminic acid, respectively, whereas dcIV and dcVII were α/β C-glucofuranosides of kermesic acid [50]. HPLC-MS analysis has also facilitated the detection and characterization of several never-before detected cochineal dye components in cochineal dyed textiles, especially O-glycosyl compounds [50, 143, 147, 152]. Species-specific anthraquinone markers were also identified in madder extract and dyed textiles, offering the possibility to distinguish *Rubia* species used for dyeing [32, 141].

Petroviciu and co-authors [125, 128, 177, 178, 185, 186] published a series of papers on the applicability of LC-MS alone and in combination with DAD detection for identification of

historical dyes including anthraquinones in historical textiles. To test the possibility of using LC–MS as a stand-alone technique for identification of dyes in historical textiles and for prediction of the naturally occurring sources they developed a systematic ESI-MS protocol in negative ion monitoring mode [128]. Through preliminary LC–MS experiments on standard dye compounds, reference natural dyes and dyed samples they suggested a series of systematic analysis steps (Fig. 14):

(i) preparation of the acid hydrolysed extract from the fiber(s) and chromatographic separation of the extracted components simultaneously using DAD and full scan-single MS stage detection systems followed by MS data processing with Automated Mass Spectral Deconvolution and Identification System (AMDIS) software;

(ii) data processing by ion extraction chromatogram (IEC) according to m/z values of the molecular ions of dyes contained in the database and correlation with retention/UV–Vis spectral data;

(iii) reinjection of the sample and using a single stage MS detection in the single ion monitoring (SIM) or multiple ion detection (MID) mode, especially for minor compounds and correlation with data about other compounds from the same biological source; and

(iv) re-injection of the sample and unambiguous confirmation of the components (especially those existing as traces) through tandem MS (SRM/MRM/product ion scan) detection.

The protocol allowed characterization of ten anthraquinone dye compounds from two biological sources (*Dactylopius coccus* Costa and *Rubiatinctorum* L.) in a red silk thread from a document dated to 1615–1616 in the National Military Museum in Bucharest. Note that several modern instruments allow for an automatic selection of the most intense ions in the chromatogram, which are subjected to fragmentation and scanning (product ion scan) in

the same analytical run. This approach has been successfully employed to detect anthraquinones in icon paintings [187].

3.4.4 HPLC based distinction between closely related insect and plant anthraquinone dyes

The identification of dyes sources in historical textiles becomes extremely complex when it comes to distinguishing between closely related biological sources with the same major dye components differentiated only by relative amounts of different chemical markers, for example, identification of exact biological source of cochineal (Mexican, Armenian, Polish etc.) and madder (*tinctorum*, *peregrina*, *cordifolia* etc). A major advantage of HPLC is that it provides quantitative data of analytes and thus a means to identify the exact biological source of anthraquinone dyes by comparing the relative composition of identified components [13, 151]. Wouters and Verhecken [33, 188] found that dcII component in American cochineal is higher than in Armenian or Polish cochineals. They proposed a graphical system based on HPLC-DAD results for distinguishing between different species of cochineal by plotting relative %age of dcII against %age of flavokermesic acid + kermesic acid, which they demonstrated by distinguishing *Dactylopius coccus, Porphyrophora polonica, Porphyrophora hamelii* in freshly prepared reference dyed samples. However, the authors cautioned that aging effects in historical textiles might change the ratios of the components for identification of the species involved, leaving the designation of dye compositions to one or more dye source(s) open to further correction since other species or mixtures of two or more dyes might have been used to produce actual dyeings. Berghe [189] slightly modified the procedure in order to improve the accuracy of the calculations for detection of cochineal species in reference dyed and historical wool and silk. In reference dyed wools, the glucoside of flavokermesic acid (fk-glu) was detected in both types of cochineal with clearly distinguishable relative amounts (max. of 0.5% in Armenian and 1.5% – 4.5% in Mexican

Cochineal). No fk-glu was detected at all in silk dyed with Armenian cochineal, while with Mexican cochineal between 0.5 and 4% of the glucoside is found. They interpreted composition of all 126 Turkmen wool and silk samples where cochineal dyeing was identified by the presence of carminic acid as the principal dye compound.In wool samples, the cochineal species was confirmed in almost all, except a few with very low or almost no fk-glu combined with very small amounts of fk+ka. For silk, interpretation of the specific insect species remained less straightforward. Turkmen silk samples, in which the fk-glu compound was present, were most probably dyed with Mexican cochineal, but for the other samples without any fk-glu, the match with either one of the two considered species is possible.

The relative ratio of the HPLC peak areas of alizarin and purpurin is often used to identify the exact biological source of madder dyes [32, 190, 191].Mouri and Laursen[32] reported a detailed HPLC-DAD-MS based identification of marker anthraquinones that permits differentiation of common species of madder used for dyeing in Eurasia and madder-dyed textiles. *R. akane*, *R. tinctorum* and *R. cordifolia/R. peregrina* could be easily distinguished from each other as they contain 6-hydroxyrubiadin, alizarin and purpurin, respectively, as characteristic markers. *R. cordifolia* and *R. peregrina*dyed textiles contain mostly purpurin, munjistin and pseudopurpurin, but little or no alizarin or 6-hydroxyrubiadin. However, a reliable marker to distinguish *R. peregrina* from *R. cordifolia* has not been identified. Rubiadin is also suggested as a marker compound to distinguish *R. peregrina* L. from *R.tinctorum* L. However, rubiadin is not a reliable marker for the identification of *R. peregrina* L. because it occurrs in varying amounts in textiles dyed with other species of madder. Very recently, Tamburini [192] presented HPLC-MS based distinction between dyer's madder (detection of ruberythric acid, lucidin primveroside and alizarin with high relative abundances compared to the other anthraquinones) and Indian madder (high relative

abundances of rubiadin and munjistin and the absence of alizarin) in Chinese textiles from Dunhuang. Karapanagiotis et al. [190] employed HPLC-DAD to identify exact madder and cochineal dye sources in ancient Egyptian fabrics of Roman, Byzantine and Islamic period periods of the Fill-Trevisiol collection. Based on semi-quantitative results from HPLC peak areas, madder dyes which were rich or poor in alizarin compared to purpurin were identified respectively as *R. tinctorum* L. and *R. peregrina* L.. Out of five samples identified as dyed with one source of coccid dyes, the absence of carminic acid in three samples indicated that these were dyed with kermes, while biological sources in two other samples were chemically identified as *Porphyrophora hamelii* Brandt (identified by comparing HPLC peak area ratio of kermesic acid to carminic acid) and *Kerria lacca,* Kerr. Similarly, Lech et al. [151] identified Polish cochineal in red fibers from chasubles from the Wawel Cathedral collection by comparing relative composition of kermesic acid and carminic acid.

Comparison of the relative composition of significant chromatographic peaks is a widely accepted approach, however, it often offers inconclusive results. New soft extraction protocols and HPLC-MS identification of previously unknown minor marker compounds in Polish, Armenian and American cochineal provides new tool for distinction between them [7, 145, 147, 151, 152]. The application of statistics on analytical data represents a powerful tool in discriminating between closely related anthraquinone sources, especially when identification of unknown samples relies on plots previously prepared with many reference samples (historical reproductions and artificially-aged specimens) [51, 54]. The combination of HPLC analysis and multivariate data analysis constitutes an excellent tool in the analysis of cochineal insects and the red dyes used in historical textiles [51]. By combining partial-least squares discriminant analysis (PLS-DA), with the qualitative and quantitative interpretation of chromatographic results it is possible to obtain accurate classifications of cochineal species present in historical textiles [54]. Serrano et al. [51] analysed 7 cochineal

species and 63 historical cochineal insect specimens using HPLC-DAD/MS^n analysis in combination with a PCA, then compared them with the results for 15 historical textiles to assess their applicability to complex real samples. By applying the developed method, they identified *Porphyrophora* sp. as specific biological source in almost all the tested historical textiles. Santos et al. [57] created a reference database using HPLC analysis for lac-dye insects (*Kerria* and *Paratachardina* genera) by analysing 76 historical lac-dye specimens and 7 reference insect specimens, and confirmed that reds and pinks in the Guimaraes carpets were achieved using lac-dye. The results indicated *Kerria* and *Paratachardina* are easily distinguished by HPLC-DAD-MS analysis due to differences in laccaic acid content. Further they applied multivariate data analysis to differentiate sources of *Kerria* and improved the lac-dye database. PCA indicated that discrimination can be obtained according to composition.These researches demonstrate that how useful and accurate combination of chromatographic analysis and multivariate data analysis approach is for the characterization of cochineal and lac species. New methods developed in recent years by Serrano [51, 54], Lech [145, 152] and Serafini [147] offer more accurate information and are highly advisable for future research on cochineal dyes.

3.5 Gas chromatography and capillary electrophoresis

Gas chromatography (GC), a separation technique that utilizes an inert gaseous mobile phase and a liquid/solid stationary phase, is capable of separating highly complex mixtures based primarily upon differences in volatility and affinity with the stationary phase [193, 194]. GC, in combination with MS detectors, has been efficiently used in the analysis of naturally occurring anthraquinone compounds and has demonstrated the ability to simultaneously identify and quantify constituents in a single chromatographic run. The main drawback of this technique is that it can be applied only to volatile and thermally stable compounds, and thus, it

requires chemical derivatization of compounds with low volatility before analysis [195, 196]. Consequently, GC is not as commonly used as liquid chromatography for the separation of organic dyes in historical textiles due to the highly polar nature of dye compounds in complex textile matrices. However, studies have reported the application of gas chromatography coupled to mass spectrometry with these limitations overcome by derivatization of the target analytes with *N,O*-Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and/or trimethylchlorosilane (TMCS) [71] and m-(trifluoromethyl)phenyltrimethylammonium hydroxide (TMTFTH) [69, 197]. Degani and co-authors [71] demonstrated effective GC separation of silylated anthraquinones without any peak overlapping even when dye extracts contained many compounds in addition to the target dyes. They detected alizarin and purpurin together with two other anthraquinones in wool references dyed with madder, based on comparison with mass spectra of reference compounds. The TIC chromatograms of the derivatized extracts of historical samples from a 16th-century Flemish tapestry provided a preliminary indication about the presence of target dyestuff molecules through extracted ion chromatograms, and the use of madder was confirmed by the presence of alizarin and purpurin through the acquisition of the SIM chromatogram.

GC-MS with a TMTFTH derivatising agent has been successfully attempted at the Canadian Conservation Institute for the characterisation of different types of natural dye constituents including flavonoid, isoflavonoid, quinone (anthraquinone and naphthoquinone), tannin, indigoid and lichen dyes found in museum objects [69]. In addition to the identification of the colorants, this approach is also useful in determining the presence of degradation products from the dyes and substrates, non-dye marker compounds, auxiliary compounds added to the dye bath, and contaminants. The alkaline reagent extracts the dye compounds by breaking the bonds between the colorants and the mordant ions/textile substrate, and derivatizes polar functionalities, to produce compounds that can be analysed by GC–MS. Three anthraquinone

sources, namely *Rubia tinctorum*, *Relbunium* and *Galium tinctorum* were identified in an orange-red thread from a 17th-18th-century Ethiopian silk hanging (Fig. 15a), a purple thread from a Paracas burial cloth (Fig. 15b), and a red porcupine quill from an embroidery on the First Nations moccasins (Fig. 15c), respectively [69].

Capillary electrophoresis (CE) is a technique which achieves separation of the components of a mixture by an applied voltage, and the separation depends on charge and mobility of each component rather than their interaction with a stationary phase [198]. CE is a fast technique allowing the combination of short analysis times, high separation efficiency and lower consumption of chemicals than HPLC; however, the sensitivity of UV-Vis absorbance detection in CE was reported to be relatively poor compared to HPLC [70, 199]. Although CE separation of anthraquinone dyes in reference natural sources, lakes and samples from works of arts has been reported to be highly efficient [199-203], it has been rarely applied in the analysis of historical textiles [70].

4. Conclusions

In summary, as an interdisciplinary combination of analytical chemistry with physics, material sciences, technical art history, archaeology and conservation science, the field of historical dye analysis has been rapidly growing in the past forty or so years. Natural anthraquinones in historical textiles have been the central point of most of the investigations in this area and their unambiguous identification continues to represent a challenge to analytical chemists. The earliest analysis methods included identification through extractions and chemical reactions, visual colour matching, paper chromatography and spectroscopic methods in the UV-Vis and IR regions. The advent of TLC further improved analytical capability for identification of these analytes. Inception of HPLC was the most significant development, resulting in more precise and informative identification. Other separation-based techniques such as GC and CE were also successfully tested, although their use is still limited

in this area of application. HPLC coupled with suitable detectors remains the most reliable and versatile technique for these analyses, when sampling from the artefact is permitted, the risk of sample loss during preparation or analysis is accepted, and/or this analytical method is the only way to answer the research question about the artefact or dye. Recent works in this area focused on improving sampling and dye extraction protocols, achieving better chromatographic separation and identifying best suited detectors for maximizing efficiency of the method. When methods of detection are considered, DAD seems to be applied more often, because it isroutinely used in many analytical laboratories and established analytical protocols. MS detection, on the other hand, offers higher sensitivity, selectivity and the possibility for unknown compound identification. However, the destructive nature of the analyses limits the applicability of chromatographic methods to a certain extent, because they involve sampling and destruction of the sample, something that is often incompatible with the preservation of historical objects. There is a growing demand for minimally-invasive/non-destructive techniques among the cultural heritage community. In this context, application of SERS, direct mass spectrometry**,** FORS and micro-spectrofluorimetry in this area reveal promising developments. Recent efforts in SERS analysis specially focus on developing minimally invasive *in-situ* methods such as Ag-embedded polymer gel loaded with a mild micro-extractant and ‘‘dry-state’’ SERS approach which can be directly applied to the area of interest on the artefact. Despite having significantly reduced the sample size and analysis time, SERS application is still limited due to complexity of the SERS probe preparation and inability to resolve dye component mixtures. Analytical techniques based on direct mass spectrometry such as DART-TOF-MS, LDI-MS, MALDI, TOF-SIMS and LMJ-SSP MS are becoming popular as alternative minimally invasive approaches. These MS based techniques offer additional advantage when identifying chromophores, in the absence of reference compounds. However, the identification and assignment of isomeric structures is possible only to a limited extent,

through the interpretation of the fragmentation pattern. FORS and *in-situ*spectrofluorometry truly represent non-invasive methods used in this area of application; but their application is rather limited to preliminary investigation for diagnostic purposes.

With all the progress, analysis of historical anthraquinone dyes remains challenging and intriguing. Most of the available techniques mainly respond to major (or more "active" towards the used detection method) dye components. Whenever the question is about the source of dye a *Rubiaceae* family plant or scale insect, multiple choices of techniques are available. Nonetheless, if the question is about where this dye comes from, or which plant or insect was used, then coupling HPLC (or UHPLC) with mass spectrometry is often the only answer at present. In the historical identification of colorants, the unequivocal identification depends largely on the availability of dedicated reference libraries and sharing of data for the identities of the target compounds. More efforts are required for the undisputable identification of anthraquinone dyes in historical textiles, especially in the development of standardized analytical protocols combining complementary analytical techniques, and the generation of comprehensive spectral databases of anthraquinone compounds for comparing and verifying the analytical results. Although, LC-based methods are still the best choice for anthraquinone dye analysis in historical textiles, preliminary analysis through non-invasive techniques seems to be a suitable approach for designing further analytical strategies. This will help minimize sampling needs, and thus preserve the integrity of valuable cultural heritage materials.

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Table 1: Most commonly identified sources of anthraquinone dyes in historical textiles

Common name	**Scientific name**
American cochineal/Mexican cochineal	*Dactylopius coccus* Costa
Armenian cochineal/Ararat cochineal	*Porphyrophora hamelii* Brandt
Polish cochineal/Polish kermes	*Porphyrophora polonica* L.
Kermes	*Kermes vermilio* Planchon
Lac insect	*Kerria lacca* Kerr
Dyer's madder/European madder	*Rubia tinctorum* L.
Wild madder	*Rubia peregrina* L.
Indian madder	*Rubia cordifolia* L.
Japanese madder	*Rubia akane*
Munjeet	*Rubia munjista*
Lady's bedstraw	*Galium verum* L.
Al/Indian Mulberry	*Morinda citrifolia*L.
Chay root	*Oldenlandia umbellata*L.
Relbunium	*Relbunium* spp.

Table 2: Examples of SERS application in anthraquinone dye analysis in historical textiles

Investigated object	**Method**	**Sample pre-treatment/Extraction**	**SERS substrate**	**Detected anthraquinone dye**	**References**
Red thread sample from a Netherlandish tapestry (16th c.)	Extractionless, hydrolysis	HF vapours	Citrate reduced Ag colloid	Alizarin	[88]
Italian cover (late 17th c.)	Extractionless, non-hydrolysis	-	Citrate reduced Ag colloid	Purpurin	[104]
Carpet from Bursa/Turkey/ Istanbul (late 16th/early 17th c.)				Lac	
Shawl from France (1850-1900 A.D)				Carminic acid	
Red weft fiber from a pre-Columbian Peruvian Textile (800 - 1350 A.D)	Extractionless, hydrolysis	-	Citrate reduced Ag colloid	Alizarin	[92]
Red wool thread found in the Royal Tumulus of In Aghelachem, Libya (300 B.C.–350 A.D.)	Extractive	Extraction with methanolic HCl	Citrate reduced Ag colloid using poly-L-lysine and ascorbic acid as aggregating agents	Madder	[101]
Archaeological Coptic textile of Egyptian origin (6th–8th A.D.)	Extractionless, non-hydrolysis	-	Photo-reduced silver nanoparticles produced *in situ* on the fiber	Alizarin	[106]
Textiles from collection of Mariano Fortuny (1871–1949)	Extractionless, non-hydrolysis	-	Ag colloidal paste prepared from citrate-reduced Ag colloids	Carminic acid	[91]
Pre-Columbian textile	Minimally invasive extraction	Ethanol wetted Ag-agar gel placed in contact with the textile surface	Ag-agar gel prepared from citrate-reduced Ag colloids	Alizarin	[110]
Feathered bag from Peru (15th–	Extractionless,	HF vapours	Citrate reduced Ag colloid and	Madder	[114]

early 16th c.)	hydrolysis		microwave-supported glucose reduction of silver sulphate in the presence of sodium citrate as a capping agent		
Cap with feathers from Chile (10th–14th century)				Madder	
Tunic from Peru (ca. 135–525)				Madder	
Tasselled tunic from Peru (1100–1250)				Carmine	
Painted cloth from India (Late 18th–early 19th c.)				Lac	
Chinese Ningxia textiles (18th–19th centuries)	Extractionless, hydrolysis	HF vapours	Citrate reduced Ag colloid	Lac	[102]
Caucasian Kaitag textiles (17th–18th c.)				Madder	
Pre-Columbian Peruvian tunic	Extractionless, hydrolysis	HF vapours	Microwave-induced glucose reduction of silver sulphate in the presence of sodium citrate	Pseudopurpurin	[103]
Pre-Columbian textiles	Minimally invasive extraction	Ethanol wetted or EDTA containing Ag-agar gel placed in contact with the textile surface	Ag-agar gel prepared from citrate-reduced Ag colloids	Alizarin	[109]
Pink thread of an Italian carpet dating to Renaissance	Extractive	Extraction with methanolic HCl followed by HPLC separation	Citrate reduced Ag colloid	Purpurin	[116]

Table 3. Anthraquinones and their sources identified through HPLC analysis in historical and archaeological textiles

Investigated object	Dated	Detector	Detected anthraquinones																			Identified anthraquinone sources								Ref
			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	Rs	Ga	Re	Mo	Cr	Co	Kr	La	
Coptic textiles, Spanish bishop tunic and Belgium tapestry	3rd c. BC – 15th c. AD	UV-Vis	+	+		+		+					+	+																	[13]
Historical textiles from different sources	7th - 17th c.	UV-Vis	+	+									+	+	+	+	+			+	+	+	+					+	+	+	+	[33]
Greco-Roman Period Fabric in Egypt	-	UV-Vis, MS	+																				+				+				[176]
Pre-Columbian Peruvian Textiles	300 BC - 1532 AD	DAD	+											+									+						+		[45]
Gobelins furniture tapestries	18th c.	DAD	+		+								+										+					+			[159]
Tapestry fibres	17th c.	DAD	+	+																											[126]
Coptic textiles	4th-12th c.	DAD	+	+									+			+	+						+					+		+	[142]
Coptic textiles	-	DAD, MS	+	+	+																										[133]
Coptic textiles	-	DAD, FLD, MS	+	+	+			+					+			+	+						+					+		+	[134]
Gallo-Roman textiles	1st-2nd c.	DAD	+	+																			+	+							[161]
Pazyryk burial textiles	500-200 BC	DAD, MS	+	+									+	+									+					+	+		[127]
Hallstatt textiles	800–400 BC	DAD											+	+																	[11]
Scottish textiles excavated from peat	16th-17th c.	DAD	+	+									+										+					+			[137]

bogs																							
Aubusson tapestry	19^{th} c.	DAD, MS	+	+	+												+						[143]
Textiles from the Holy Mountain of Athos	15^{th}-19^{th} c.	DAD	+	+						+	+	+	+				+			+			[136]
Fragment of an Egyptian garment	7^{th} or 8^{th} c.	DAD	+	+								+	+	+	+	+	+					+	[169]
Aubusson tapestry	19^{th} c.	DAD, FLD	+	+	+												+						[175]
Ancient textiles from Xinjiang	About 1000 BC	DAD, MS	+	+													+						[10]
Textile fragment from pre-Columbian mummies	-	DAD	+	+	+					+			+					+	+	+			[144]
Mamluk textiles	1250-1517 AD	DAD	+	+			+		+	+	+	+	+				+			+		+	[146]
Scandinavian peat bog textiles	Early Iron Age	DAD	+	+													+						[156]
Wool thread from the Royal Tumulus of In Aghelachem	300-350 A.D.	DAD	+	+													+						[102]
Andean textiles	200 BC-AD 1476	DAD		+		+											+						[76]
Silk thread	1615–1616 AD	MS	+	+	+		+	+		+	+	+					+			+			[128]
Textiles from the Xeropotamou monastery	16^{th}-19^{th} c.	DAD								+	+	+								+			[149]
Textiles from the Simonos Petra monastery	16^{th}-20^{th} c.	DAD, MS	+	+						+	+	+					+			+			[177]
Epitaphios from	15^{th} c.	MS									+	+									+		[181]

SucevițaMonastrey																				
Italian velvet brocades	15th c.	DAD, MS								+	+	+		+	+	+		+			[151]
Historical textiles (Italy, Iran, Turkey, India) from museum collections in Portugal	15th-19th c.	DAD, MS								+	+	+		+	+	+		+			[51]
Historical Chinese silk fragments	-	DAD, MS	+	+													+				[154]
Ottoman period silk brocade	16th c.	DAD								+								+			[170]
Fustatcarpet fragments	8th – 18th c.	DAD	+	+			+						+				+			+	[150]
Caftan garment from Topkapi Palace Museum		DAD								+								+			[132]
Historical textiles from Japan, Iran, China, Tibet and Mongolia	200-3000 years old	DAD, MS	+	+		+		+									+				[32]
Historical textiles from Romanian collections	15th-20th c.	DAD, MS	+	+	+		+	+	+	+	+	+		+			+	+	+		[178, 185]
Red silk thread	16th c.	MS	+	+	+			+	+			+	+				+			+	[186]
Ancient Kaitag textiles from Caucasus	-	DAD	+	+													+				[115]
*Red silkfrom*Simonos Petra monastery	18th-19th c.	DAD, MS								+	+	+		+	+	+		+			[50]
Decorative cloth from the church of Bosco Marengo	16th-17th c.	DAD, MS								+				+				+			[72]

Late Bronze Age textile from the Yanghai archaeological site	-	DAD, MS	+	+			+				+											+									[6]
Arraiolos carpets	17th-19th c.	DAD, MS	+	+	+							+	+								+	+	+			+		+			[43]
Chehrabad salt mine textiles	About 2000 years old	DAD, MS	+	+																			+								[157]
Ottoman Silk Brocades	16th-17th c.	DAD	+	+									+		+					+			+					+	+		[131]
Pre-Columbian Peruvian Textiles	1200–1532 AD	MS											+	+	+					+	+	+						+			[145]
Pre-Hispanic textiles	400-1450 AD	DAD		+																					+						[46]
European and Asian historical textiles	15th-18th c.	DAD, MS											+	+	+	+	+	+	+		+	+						+	+		[54]
Persian/Safavid and Indian/Mughal period velvets	16th -18th c.	DAD	+	+									+			+			+				+					+		+	[158]
Guimaraes carpets	16th-17th.	MS														+	+	+	+											+	[57]
Ningxia carpets	17th-19th c.	DAD	+	+						+						+							+							+	[113]
Noin-Ula textiles	1st c.	DAD	+	+				+					+	+			+						+				+	+	+	+	[139]
Historical red textiles from Warsaw National Museum	16th-19th c.	DAD, MS											+	+	+					+	+	+							+		[152]
Wari textiles from Huaca Malena archaeological site	7th -11th c.	DAD											+															+			[204]
Turkmen Weavings	-	DAD	+	+					+				+	+	+	+			+	+			+					+		+	[205]
Italian carpet	14th-17th c.	DAD/SERS		+										+	+								+	+				+			[116]

Coptic Textiles	-	DAD, MS	+	+																	+												[179]
Coptic textiles	-	DAD, MS	+	+	+		+					+	+	+	+						+								+				[83]
Medieval silk textiles	14th-15th c.	MS	+	+					+												+	+											[180]
Ancient textiles from Israel	13th-10th c. BCE	DAD	+	+		+	+														+												[153]
Red silk threads from documents seals	15th c.	DAD, MS	+	+	+		+	+	+				+	+	+						+								+				[125]
Turkey red textiles, University of Glasgow Archives	19th c.	DAD	+	+					+												+												[206]
Historical carpet from National Museum in Krakow	16th c.	MS												+	+	+													+				[207]
Egyptian fabrics of Roman, Byzantine and Islamic periods	1st -7th c.	DAD	+	+			+				+	+	+	+	+	+	+			+	+	+						+	+	+			[190]
Pacific barkcloths		DAD, MS									+													+									[47]
Pacific barkcloths		DAD	+	+						+	+										+			+									[44]
Chinese textiles from Dunhuang in British Museum's collection	7th-10th c.	MS	+	+		+	+	+						+	+	+	+				+								+				[192]
Black dyed wool from Belgian archives	1650-1850	DAD	+	+	+		+	+	+			+									+							+					[160]
Scottish wool tartans	18th c. - 1822	DAD, MS									+		+	+	+	+		+	+	+								+			+		[208]

European wool and silk tapestries	16th–18th c.	DAD, MS	+	+	+		+	+		+	+	+	+		+		[209]

Note: Rs – *Rubia* spp., Ga- *Gallium* spp., Re – *Relbunium* spp., Mo – *Morinda* spp., Cr – Chay root, Co – Cochineal, Kr – Kermes, La - Lac

Table 4: Absorbance maxima of major anthraquinones found in historical textiles

Anthraquinones	**Absorbance Maxima (nm)**	**Ref.**
Alizarin, **2**	249, 280, 431	[141]
Purpurin, **3**	256, 295, 480	[141]
Xanthopurpurin, **4**	200, 245, 281, 416	[39]
Pseudopurpurin, **5**	258, 494	[153]
Rubiadin, **6**	203, 244, 278, 410	[39]
Munjistin, **7**	247, 285, 418	[153]
Anthragallol, **8**	208, 245, 283, 414	[39]
Galiosin	201, 255, 288, 434	[39]
Lucidin, **9**	203, 245, 280, 414	[39]
Quinizarin, **10**	224, 250, 280, 328, 470, 515	[175]
Morindone, **11**	247, 266, 407	[43]
6-hydroxyrubiadin	278, 344, 429	[141]
Lucidinprimeveroside	203, 265, 406	[39]
Ruberythric acid	199, 261, 334, 418	[39]
Rubiadinprimeveroside	203, 269, 412	[39]
Carminic acid, **12**	275, 309, 493	[50]
Kermesic acid, **13**	223, 273, 307, 489	[50]
Flavokermesic acid, **14**	223, 285, 429	[50]
2-C-glucopyranoside of flavokermesic acid (DCII), **20**	221, 290, 431	[50]
4-aminocarmicic acid (DCIII)	273, 525, 561	[50]
3,4-dideoxycarminic acid (DDCA)	198, 275, 492	[50]
2-C-α-glucofuranoside of kermesic acid (DCIV)**21**	225, 275, 311, 491	[50]
2-C-β-glucofuranoside of kermesic acid (DCVII), **22**	223, 277, 309, 491	[50]
Laccaic acid A, **15**	285, 491	[136]
Laccaic acid A, **15**	287, 225, 490	[133]
Laccaic acid B, **16**	225, 287, 490	[133]
Laccaic acid C, **17**	225, 287, 490	[133]
Laccaic acid E, **18**	225, 287, 485	[133]
Erythrolaccin, **19**	264, 294, 464	[59]

Table 5: Fluorimetric characteristics of the anthraquinone compounds in chromatographic system[174]

Compound	**Native fluorescence detection optimum [nm]**	**Fluorescence detection optimum with post-column complexation with zirconyl [nm]**
Carminic acid	Ex: 275, Em: 580	Ex: 285, Em: 580
Anthragallol	No fluorescence	No fluorescence
Alizarin	No fluorescence	Ex: 265, Em: 595
Quinizarin	Ex: 240, Em: 535	Ex: 245, Em: 535
Xanthopurpurin	Ex: 250, Em: 535	Ex: 250, Em: 540
Purpurin	Ex: 255, Em: 545	Ex: 270, Em: 565

Ex – Excitation; Em – Emission

Figure Caption

Fig. 1: Biosynthesis of anthraquinones. Adapted with permission from Ref. [28]. Copyright (2001) Kluwer Academic Publishers.

Fig. 2: Most commonly detected anthraquinones in historical textiles

Fig. 3: (a) Two Peruvian fabrics showing both their reflectance curves (----) and the curves of the H_2SO_4 solutions of the extracted dye (——). Both solutions show the characteristic double absorption maximum of cochineal. (b) H_2SO_4 (conc.) solutions from red natural dyes, which may have been used in the textiles of Pre-Columbian Peru. Adapted with permission from ref. [64], Copyright (1978) American Chemical Society.

Fig. 4. EEM spectra with a λ_{ex} of 300−600 nm and λ_{em} of 500−700 nm for: (a) the silk dyed with *akane* red (reference), (b) the red twill of the cloth lining for the mirror case, and (c) the red plain silk of the sleeveless coat for a dancer. Reprinted with permission from Ref. [73], Copyright (2009) American Chemical Society.

Fig. 5 A) Visible light image of morral 14,255. B) Visible light image of morral 7569. Yellow dots indicate the analyzed areas. C–D) Reflectance and Log(1/R) spectra of morrals 14,255 and 7569, and red dye references. Reprinted with permission from Ref. [19], Copyright (2017) Elsevier.

Fig. 6: SERS spectra of alizarin (a), purpurin (b) and carminic acid (c) extracted from the textile mock-ups by means of Ag-agar gel. Excitation at 514.5 nm. (d) SERS spectra of alizarin obtained by means of Ag-agar gel extraction from a pre-Columbian piece of textile. Excitation at 514.5 nm. Adapted with permission from Ref. [110], Copyright (2012) John Wiley and Sons.

Fig. 7: LDI daughter ion spectra of 1,2-dihydroxyanthraquinone (alizarin) for P +1 (m/z 242) from a swatch of silk dyed with madder. Adapted with permission from Ref. [118], Copyright (2017) Elsevier.

Fig. 8: HPLC chromatograms collected at 275 nm (a) for wool dyed with cochineal and (b) undyed mordanted wool, treated with HCl, TFA and oxalic acid methods. Reprinted with permission from Ref. [146]. Copyright (2009), Springer-Verlag.

Fig. 9. Chromatograms at 256 nm of archaeological red/brown sample from Dava Moor collection obtained for extracts with acidified MeOH (A) and acidified MeOH–DMF (B) methods: (1) alizarin and (2) purpurin. Reprinted with permission from Ref. [137], Copyright (2005) Elsevier.

Fig. 10. Chromatograms obtained with the 15 cm HPLC Luna column (A–D) and the 10 cm UHPLC BEH Shield column (E–H), using aqueous phosphoric acid or aqueous formic acid. The chromatographic resolution is compared for the most representative compounds (luteolin (lu), genistein (ge), alizarin (al), apigenin (ap), curcumin (cu), indigotin (in), purpurin (pu), dcII (minor compound of *D. coccus*Costa), carminic acid (ca), flavokermesic acid (fk) and kermesic acid (ka)) and other minor compounds of the mixture of dyestuff references and the mixture of insect dyes. UHPLC and HPLC analyses were carried at similar gradient elution conditions with a runtime of 40 min and a flow rate of 0.2 mL/min. The column oven temperature was set at 35 °C for the HPLC analyses, while the UHPLC analyses were carried out at 40 °C. Reprinted with permission from Ref. [163], Copyright (2013) Elsevier.

Fig. 11. HPLC-DAD chromatogram of archaeological textiles from (a) 22ndNoin-Ula barrow, Mongolia, first century AD (chromatogram at 440 nm). Adapted with permission from Ref. [138], Copyright (2016) Elsevier. (b) Timna Valley, Israel, late 2nd millennium BCE (chromatogram at 454 nm). Adapted with permission from Ref. [152], Copyright **(**2017) Sukenik et al.

Fig. 12. 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. Adapted with permission from Ref. [132], Copyright (2003) Elsevier.

Fig. 13: Proposed fragmentation pattern for xanthopurpurin (B); A, alizarin; C, monochloroalizarin. Reprinted with permission from Ref. [132], Copyright (2003) Elsevier.

Fig. 14. Flow chart diagram supporting the analytical protocol proposed for identification of dyes used in historic textiles based on MS data (qualitative estimations addressing the relative intensities of peaks are made in relation with the signal to noise ratio S/N as following: Low $3 \geq S/N \geq 10$; Moderate $10 < S/N \leq 50$; High $S/N \geq 50$). Adapted with permission from Ref. [127], Copyright (2009) Elsevier.

Fig. 15. Partial EICs (m/z 254, 268, 281, 282, 297, 298, 341) of TMTFTH extracts from (a) an orange-red thread from a seventeenth-eighteenth century Ethiopian silk hanging (22–30 minutes), (b) a purple thread from a Paracas burial cloth (22–30 minutes), and (c) a red porcupine quill from embroidery on First Nations moccasins (24–30 minutes). Reprinted with permission from Ref. [69] Copyright (2018) Taylor & Francis.

Fig. 1

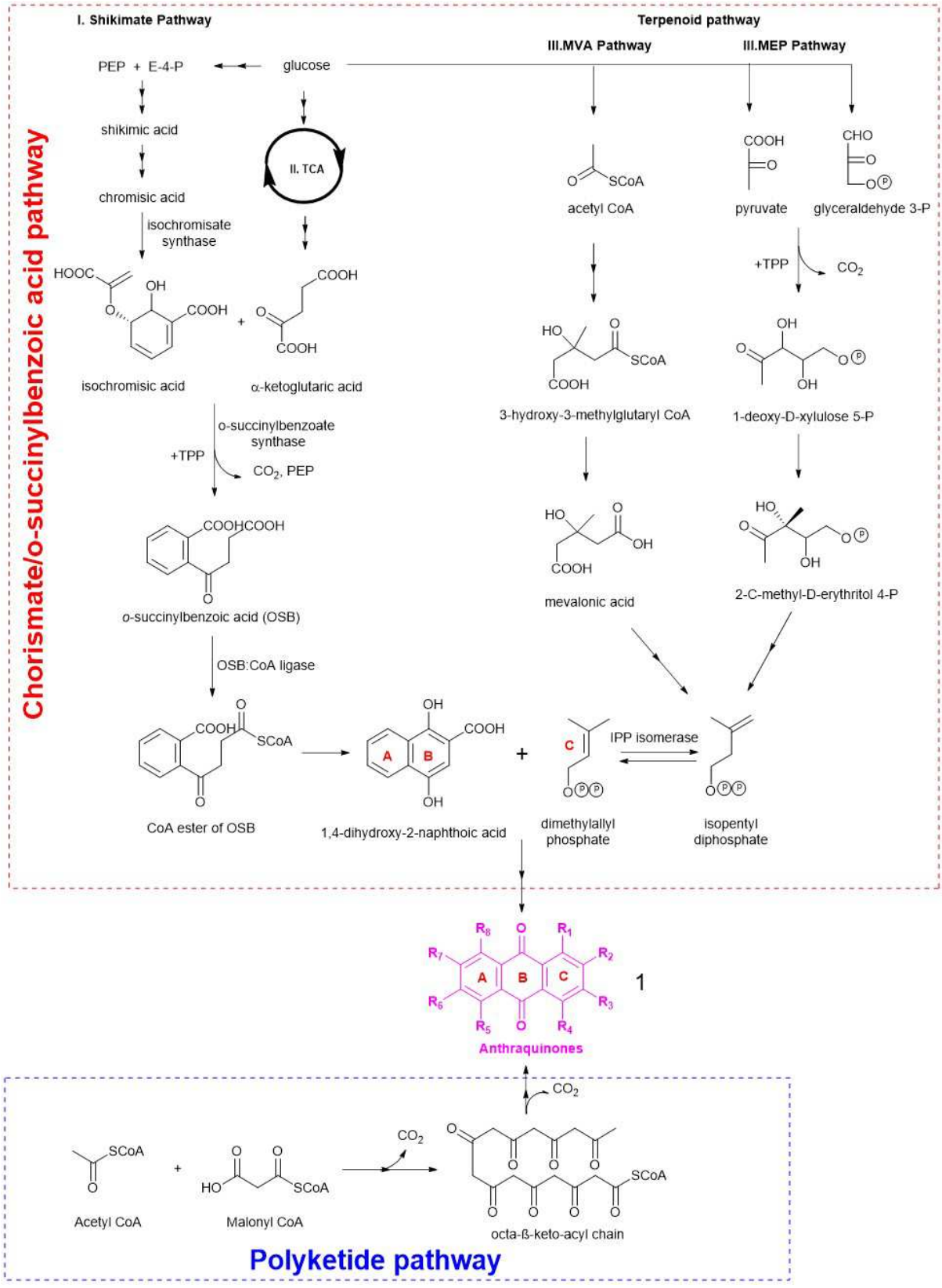

I. Shikimate Pathway
Terpenoid pathway
III.MVA Pathway
III.MEP Pathway
Chorismate/o-succinylbenzoic acid pathway
PEP + E-4-P
glucose
shikimic acid
chromisic acid
isochromisate synthase
II. TCA
acetyl CoA
pyruvate
glyceraldehyde 3-P
+TPP
CO2
isochromisic acid
α-ketoglutaric acid
3-hydroxy-3-methylglutaryl CoA
1-deoxy-D-xylulose 5-P
o-succinylbenzoate synthase
+TPP
CO2, PEP
o-succinylbenzoic acid (OSB)
mevalonic acid
2-C-methyl-D-erythritol 4-P
OSB:CoA ligase
CoA ester of OSB
1,4-dihydroxy-2-naphthoic acid
IPP isomerase
dimethylallyl phosphate
isopentyl diphosphate
Anthraquinones
1
CO2
Acetyl CoA
Malonyl CoA
octa-ß-keto-acyl chain
Polyketide pathway

Fig. 2

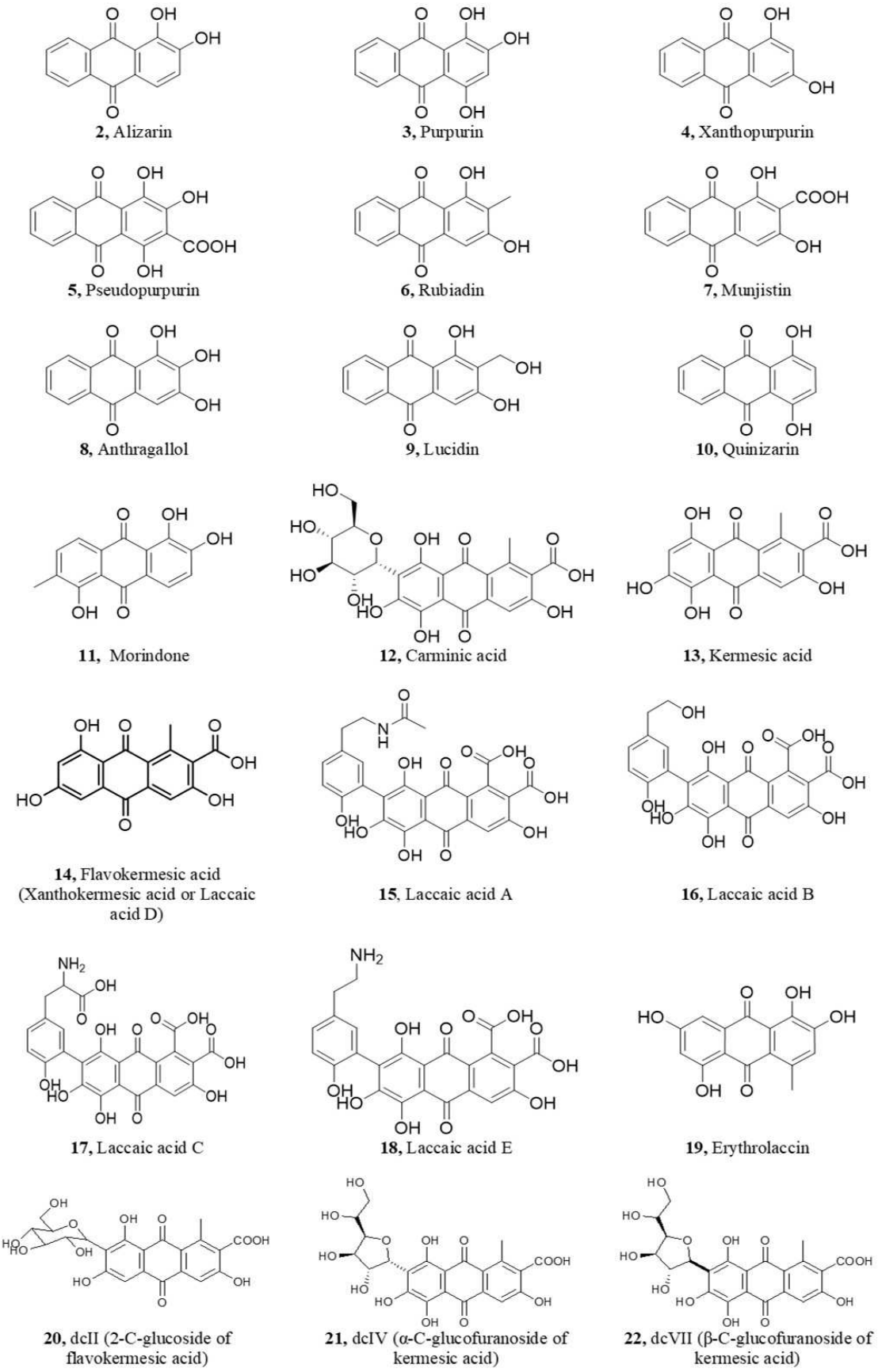

2, Alizarin
3, Purpurin
4, Xanthopurpurin
5, Pseudopurpurin
6, Rubiadin
7, Munjistin
8, Anthragallol
9, Lucidin
10, Quinizarin
11, Morindone
12, Carminic acid
13, Kermesic acid
14, Flavokermesic acid (Xanthokermesic acid or Laccaic acid D)
15, Laccaic acid A
16, Laccaic acid B
17, Laccaic acid C
18, Laccaic acid E
19, Erythrolaccin
20, dcII (2-C-glucoside of flavokermesic acid)
21, dcIV (α-C-glucofuranoside of kermesic acid)
22, dcVII (β-C-glucofuranoside of kermesic acid)

Fig. 3

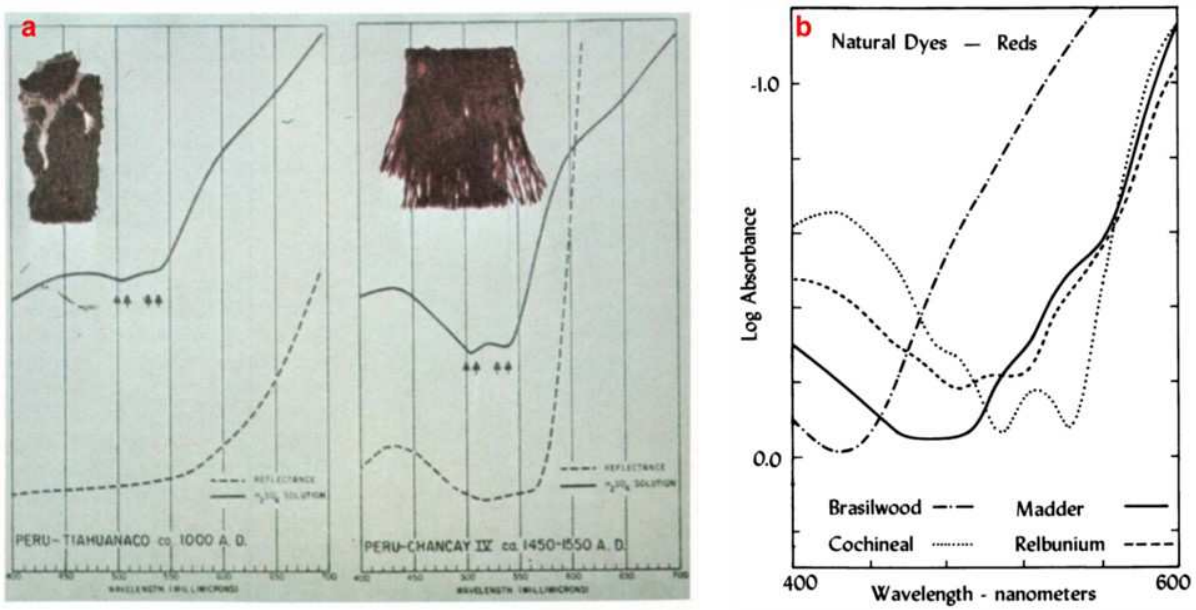

a
PERU- TIAHUANACO ca. 1000 A. D.
PERU-CHANCAY IV ca. 1450-1550 A. D.
b
Natural Dyes — Reds
-1.0
0.0
Log Absorbance
Brasilwood
Madder
Cochineal
Relbunium
400
600
Wavelength - nanometers

Fig. 4

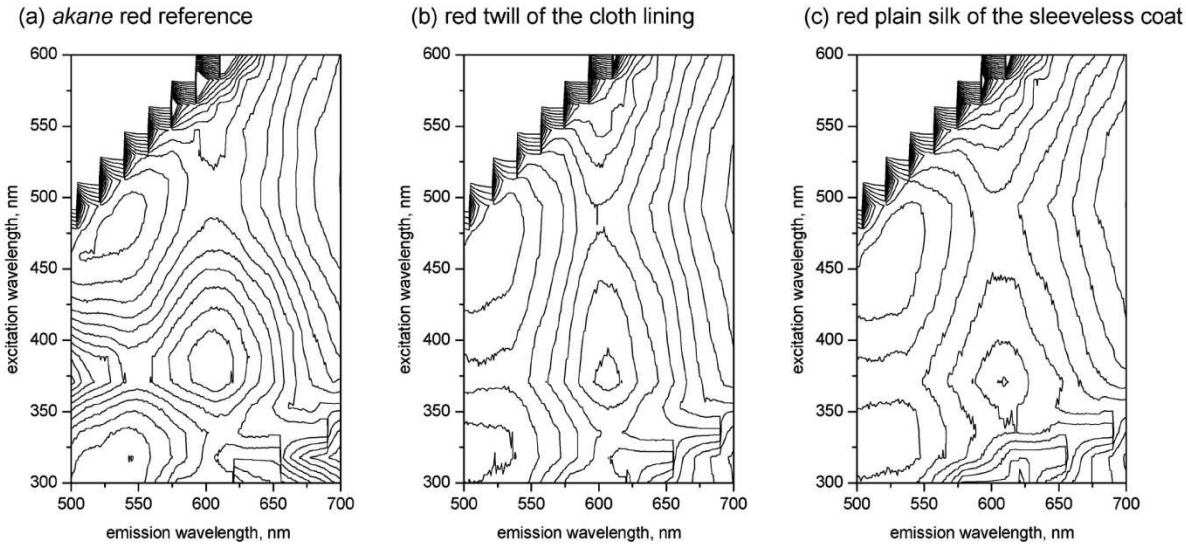

(a) akane red reference
(b) red twill of the cloth lining
(c) red plain silk of the sleeveless coat
excitation wavelength, nm
emission wavelength, nm
600
550
500
450
400
350
300
500
550
600
650
700

Fig. 5

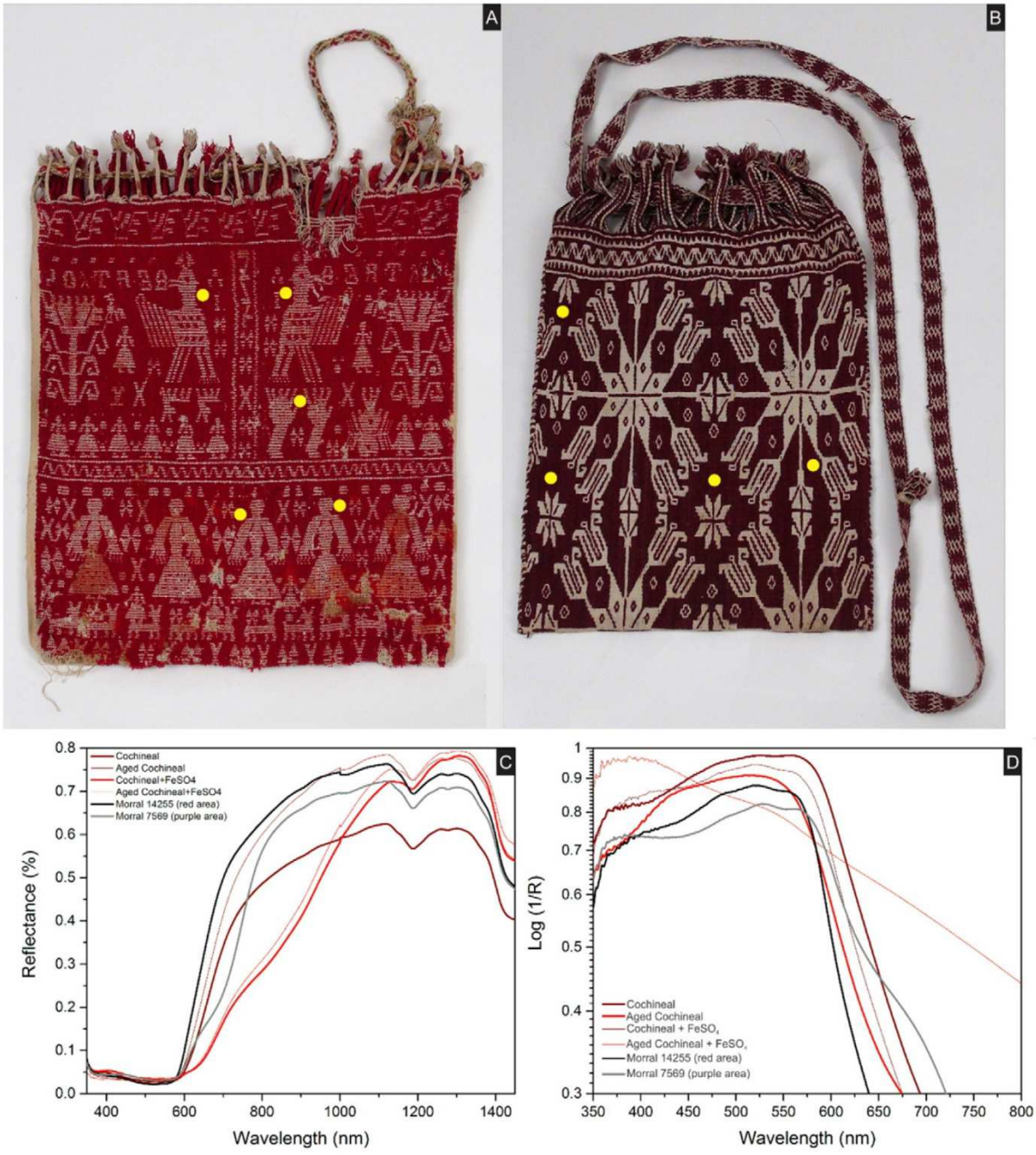

A
B
C
Cochineal
Aged Cochineal
Cochineal+FeSO4
Aged Cochineal+FeSO4
Morral 14255 (red area)
Morral 7569 (purple area)
Reflectance (%)
Wavelength (nm)
D
Log (1/R)
Cochineal
Aged Cochineal
Cochineal + FeSO4
Aged Cochineal + FeSO4
Morral 14255 (red area)
Morral 7569 (purple area)
Wavelength (nm)

Fig. 6

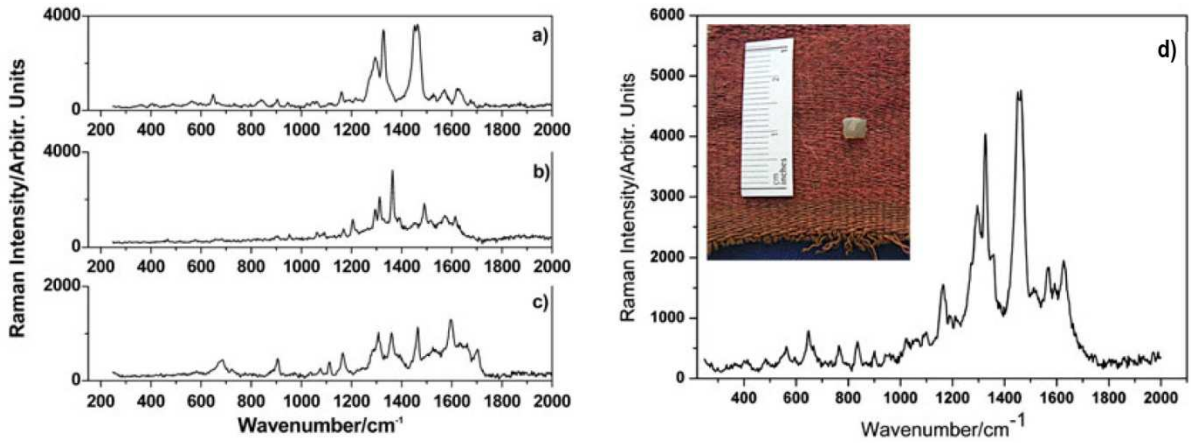

Raman Intensity/Arbitr. Units
a)
b)
c)
d)
Wavenumber/cm-1
Wavenumber/cm-1

Fig. 7

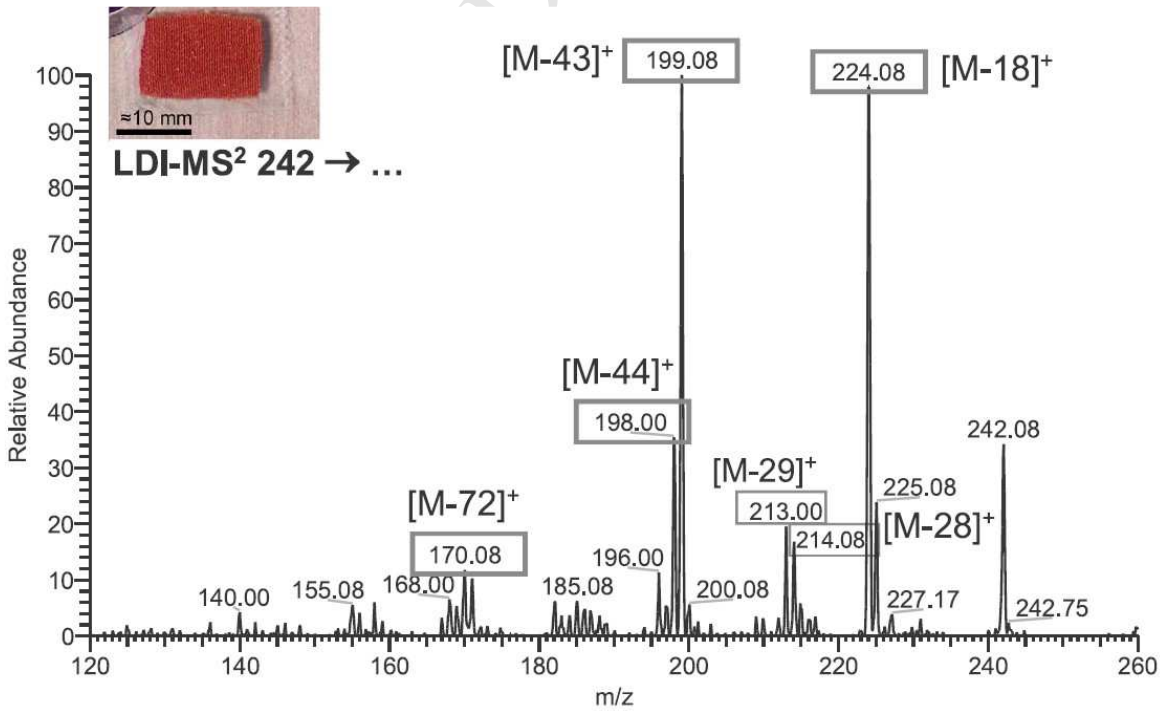

≈10 mm
LDI-MS² 242 → …
Relative Abundance
m/z
[M-43]+
199.08
224.08
[M-18]+
[M-44]+
198.00
[M-29]+
213.00
214.08
225.08
[M-28]+
242.08
[M-72]+
170.08
196.00
140.00
155.08
168.00
185.08
200.08
227.17
242.75

Fig. 8

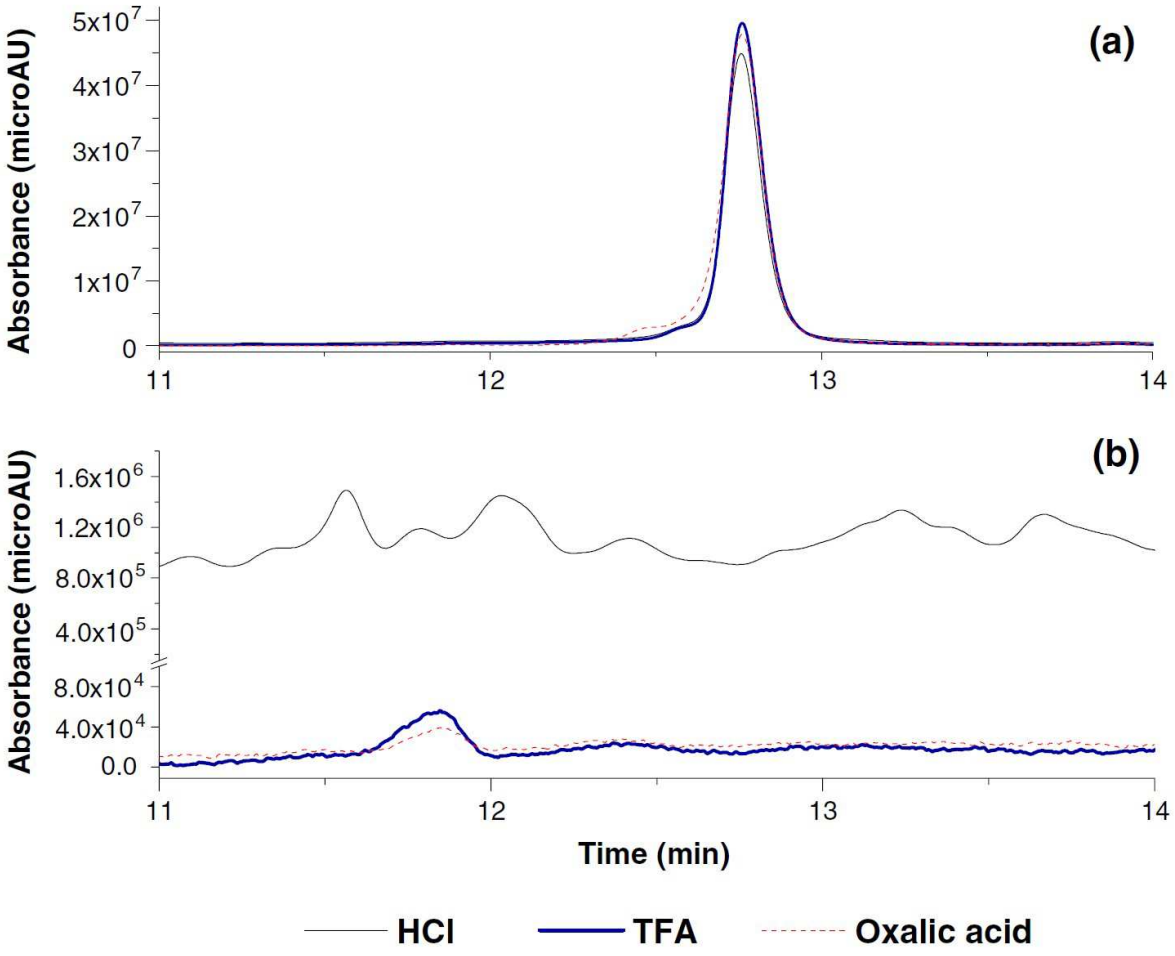


Fig. 9

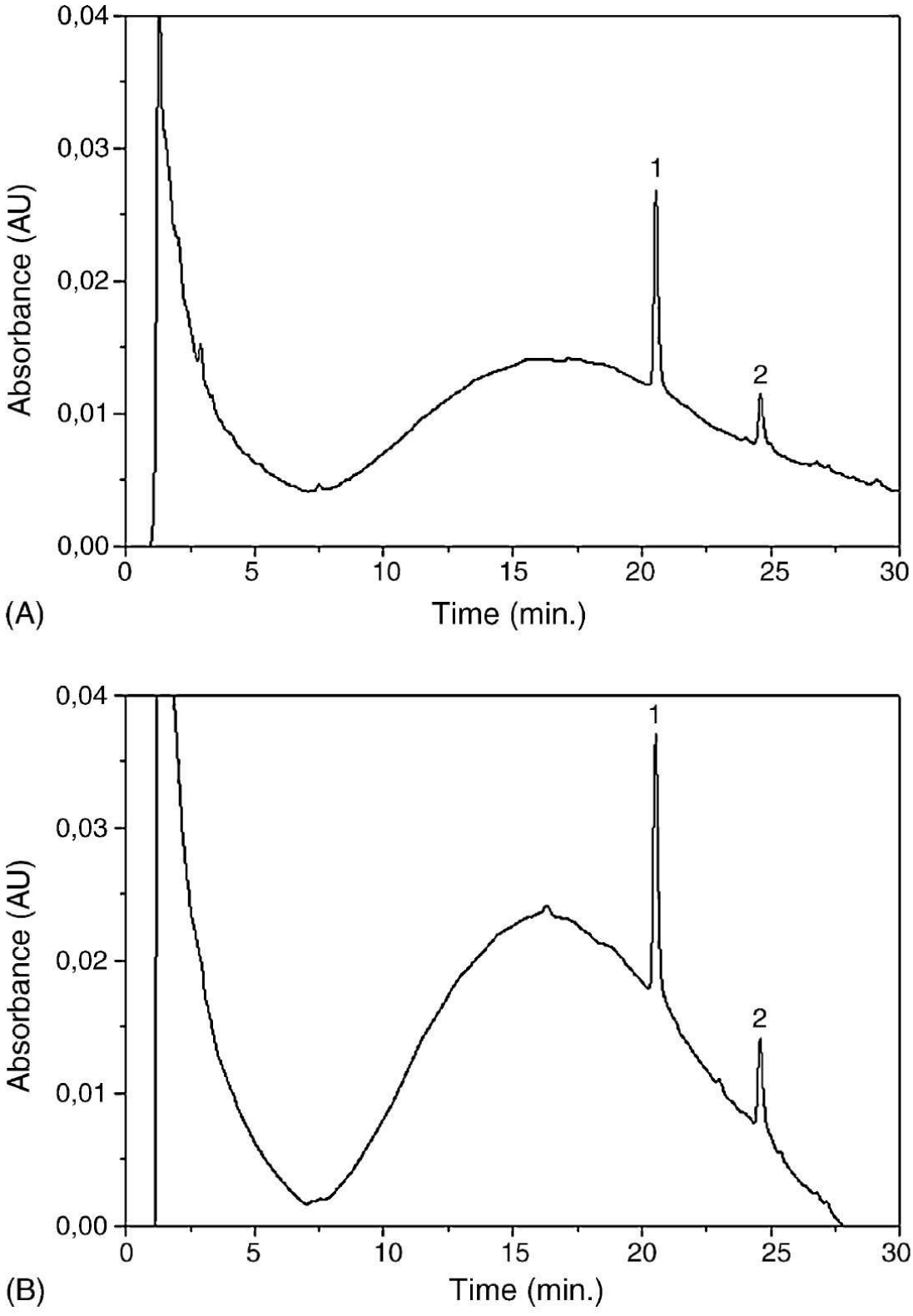

0,04
0,03
0,02
0,01
0,00
Absorbance (AU)
1
2
0
5
10
15
20
25
30
Time (min.)
(A)
0,04
0,03
0,02
0,01
0,00
Absorbance (AU)
1
2
0
5
10
15
20
25
30
Time (min.)
(B)

Fig. 10

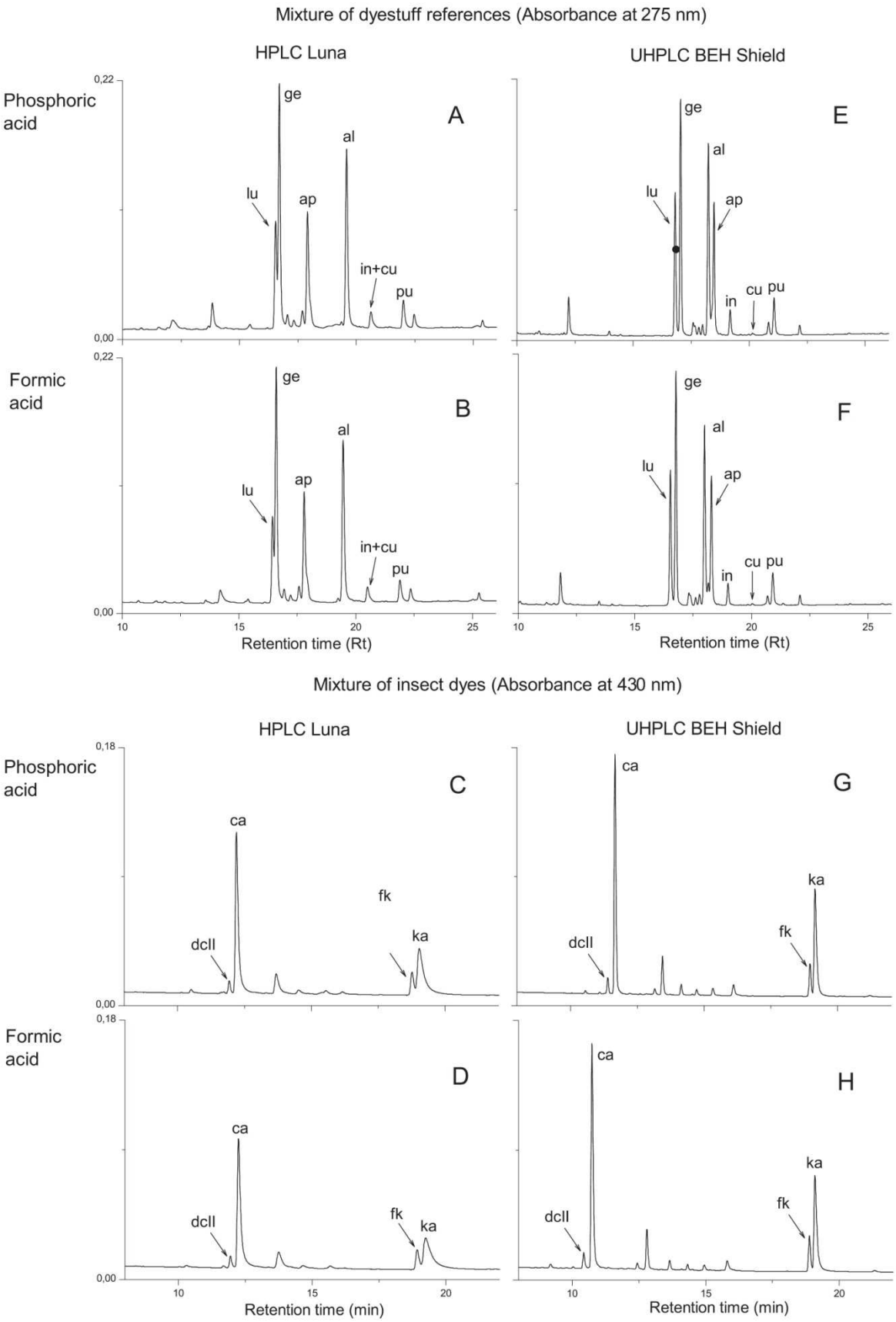

Mixture of dyestuff references (Absorbance at 275 nm)
HPLC Luna
UHPLC BEH Shield
Phosphoric acid
Formic acid
A
B
E
F
ge
al
ap
lu
in+cu
in
cu
pu
0,22
0,00
10
15
20
25
Retention time (Rt)
Mixture of insect dyes (Absorbance at 430 nm)
C
D
G
H
ca
dcII
fk
ka
0,18
Retention time (min)

Fig. 11

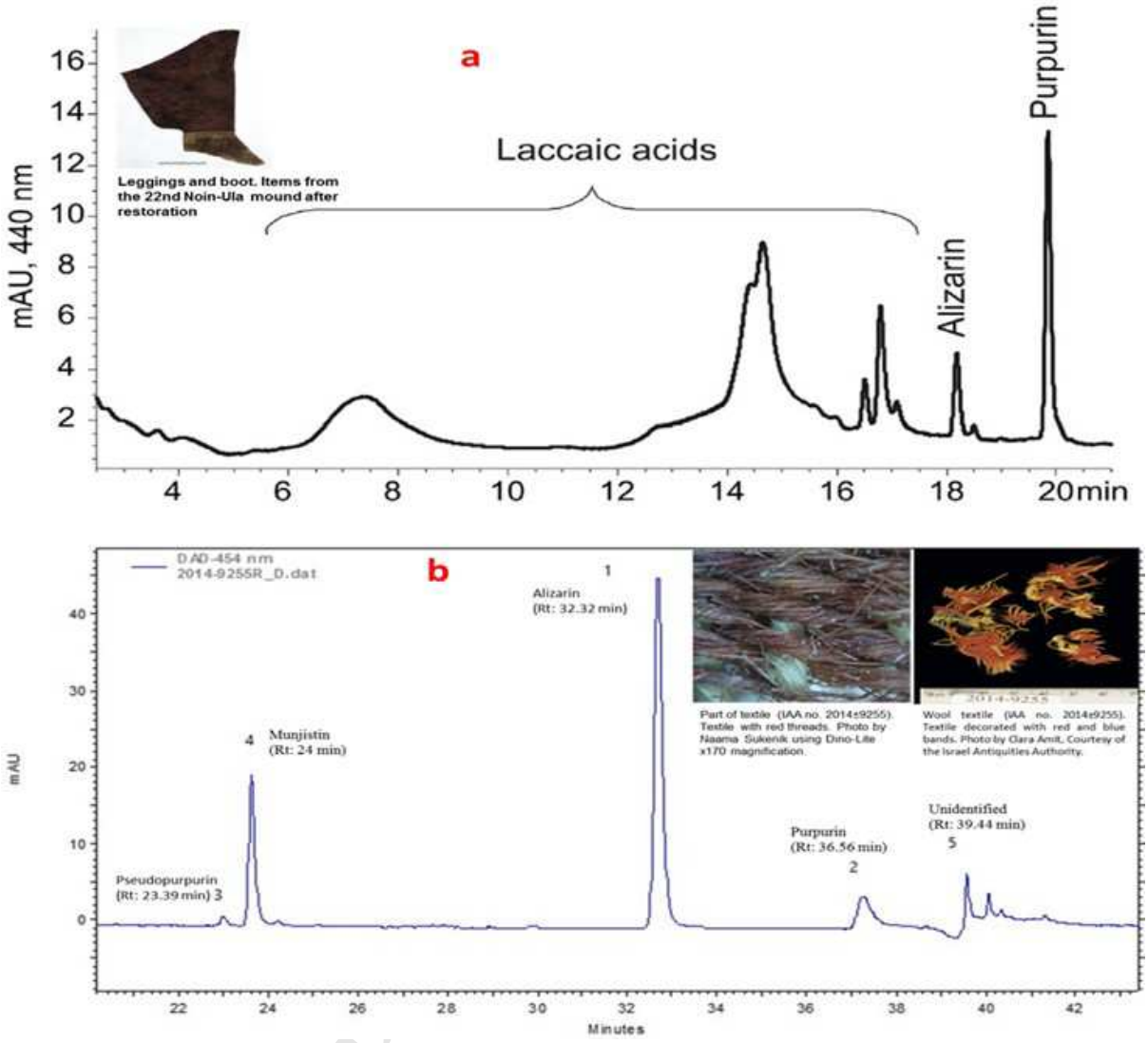

a
Laccaic acids
Alizarin
Purpurin
mAU, 440 nm
Leggings and boot. Items from the 22nd Noin-Ula mound after restoration
b
DAD-454 nm
2014-9255R_D.dat
1
Alizarin
(Rt: 32.32 min)
4
Munjistin
(Rt: 24 min)
Pseudopurpurin
(Rt: 23.39 min) 3
Purpurin
(Rt: 36.56 min)
2
Unidentified
(Rt: 39.44 min)
5
Part of textile (IAA no. 2014±9255). Textile with red threads. Photo by Naama Sukenik using Dino-Lite x170 magnification.
Wool textile (IAA no. 2014±9255). Textile decorated with red and blue bands. Photo by Clara Amit, Courtesy of the Israel Antiquities Authority.
mAU
Minutes

Fig. 12

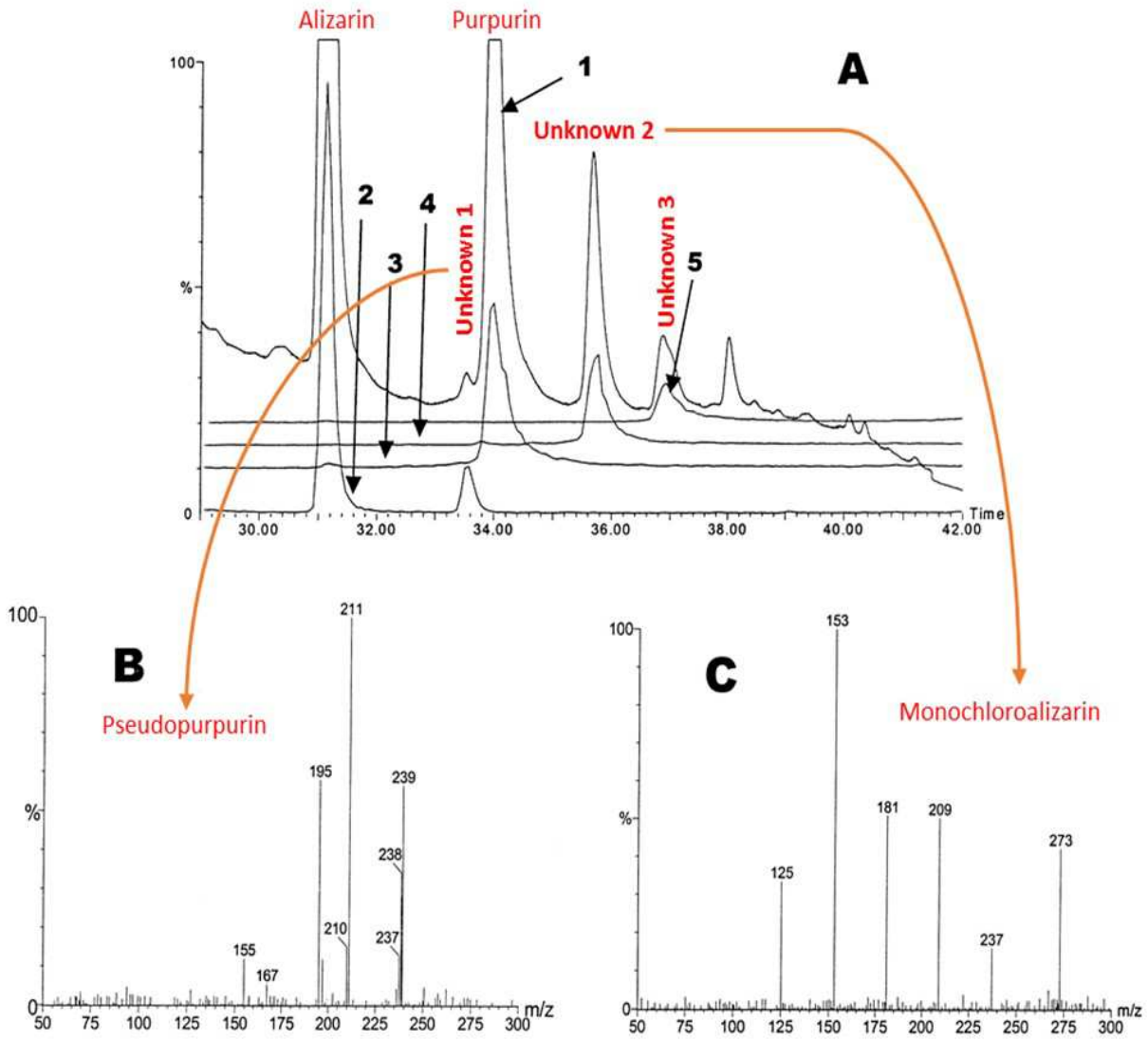


Fig. 13

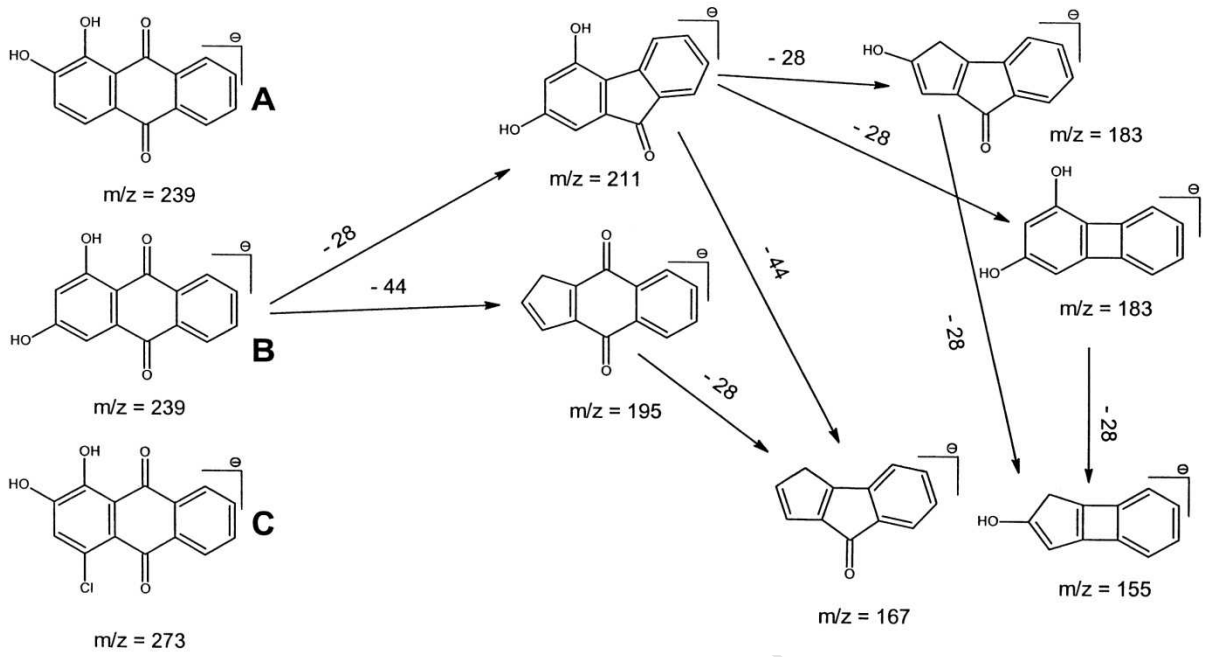


Fig. 14

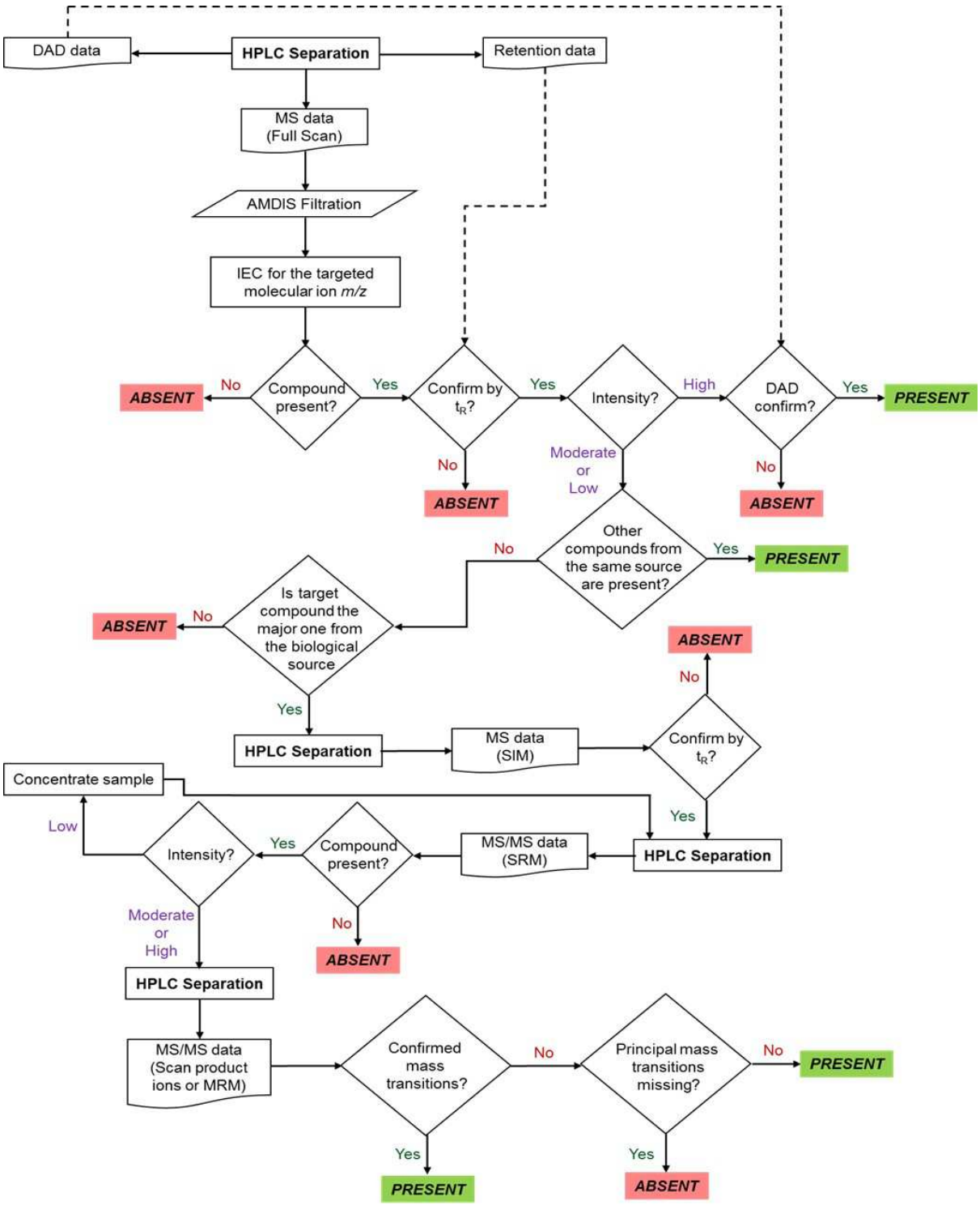
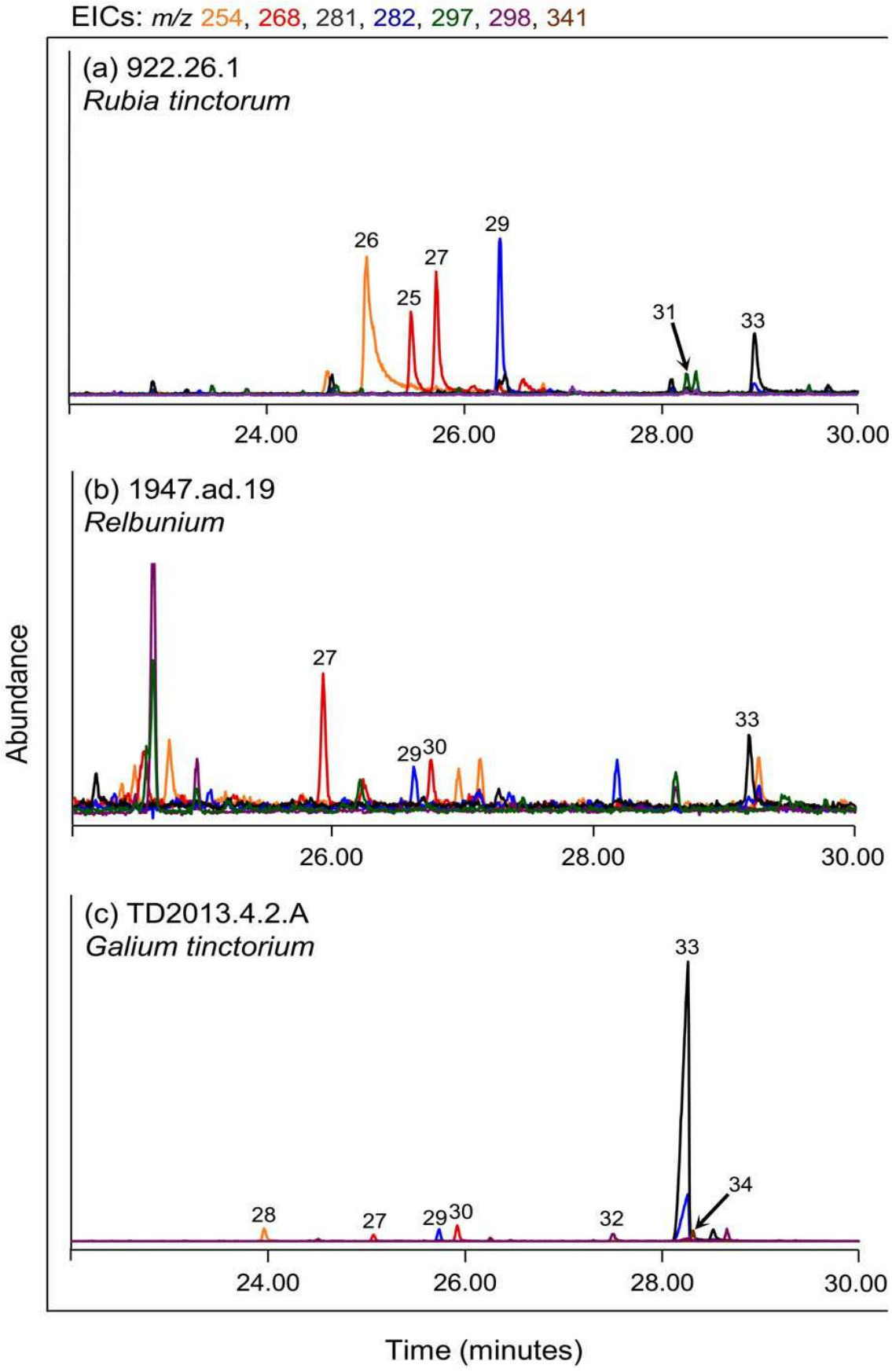
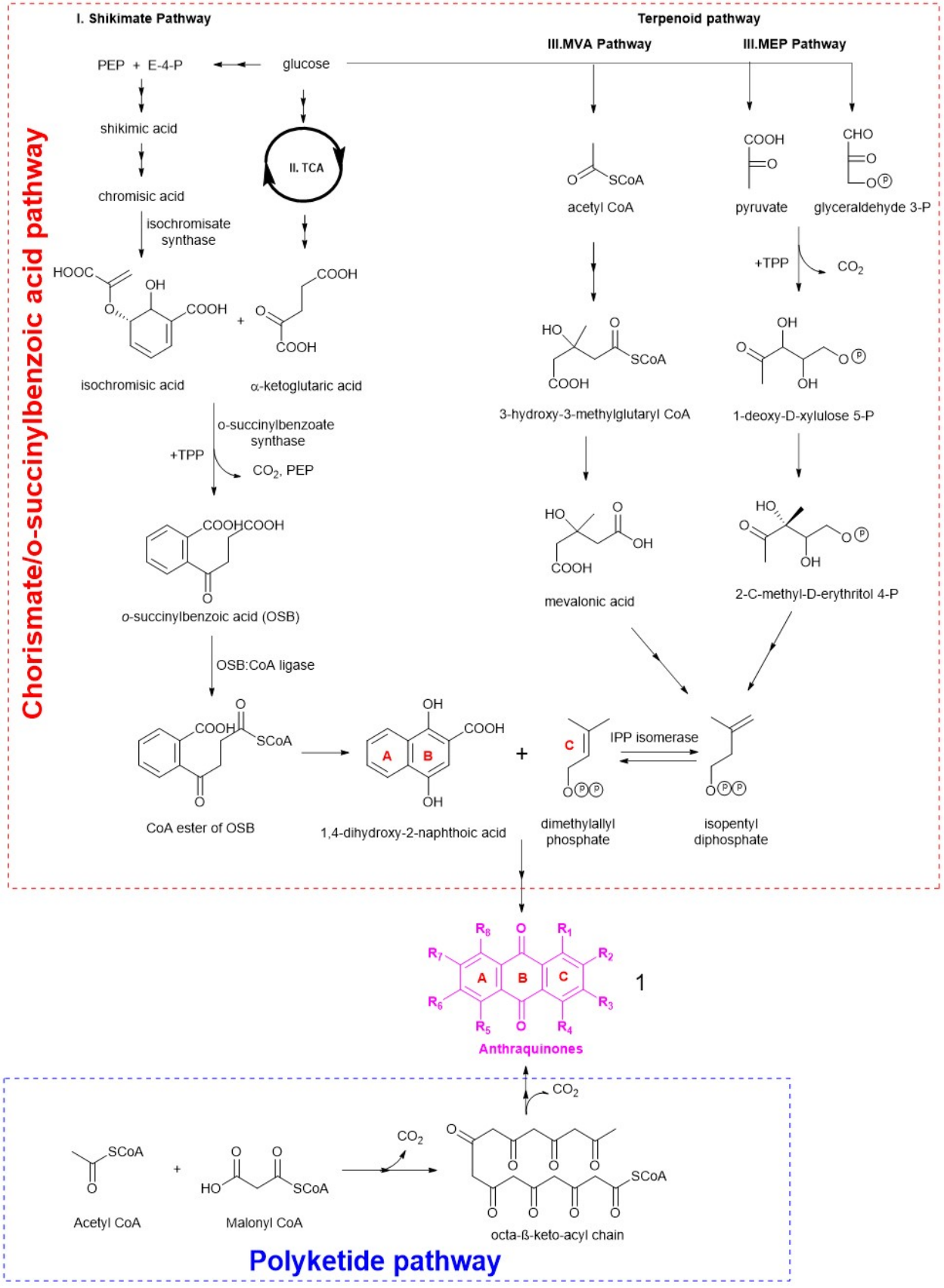
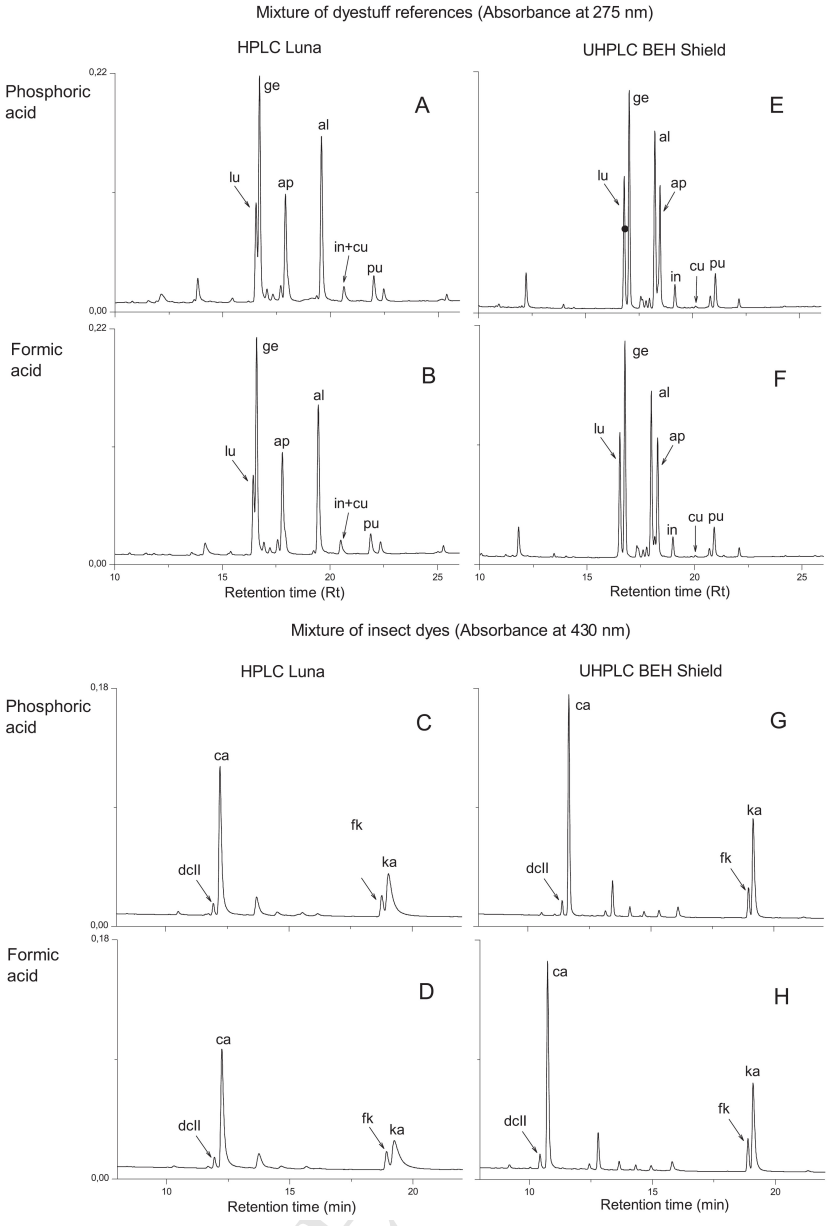
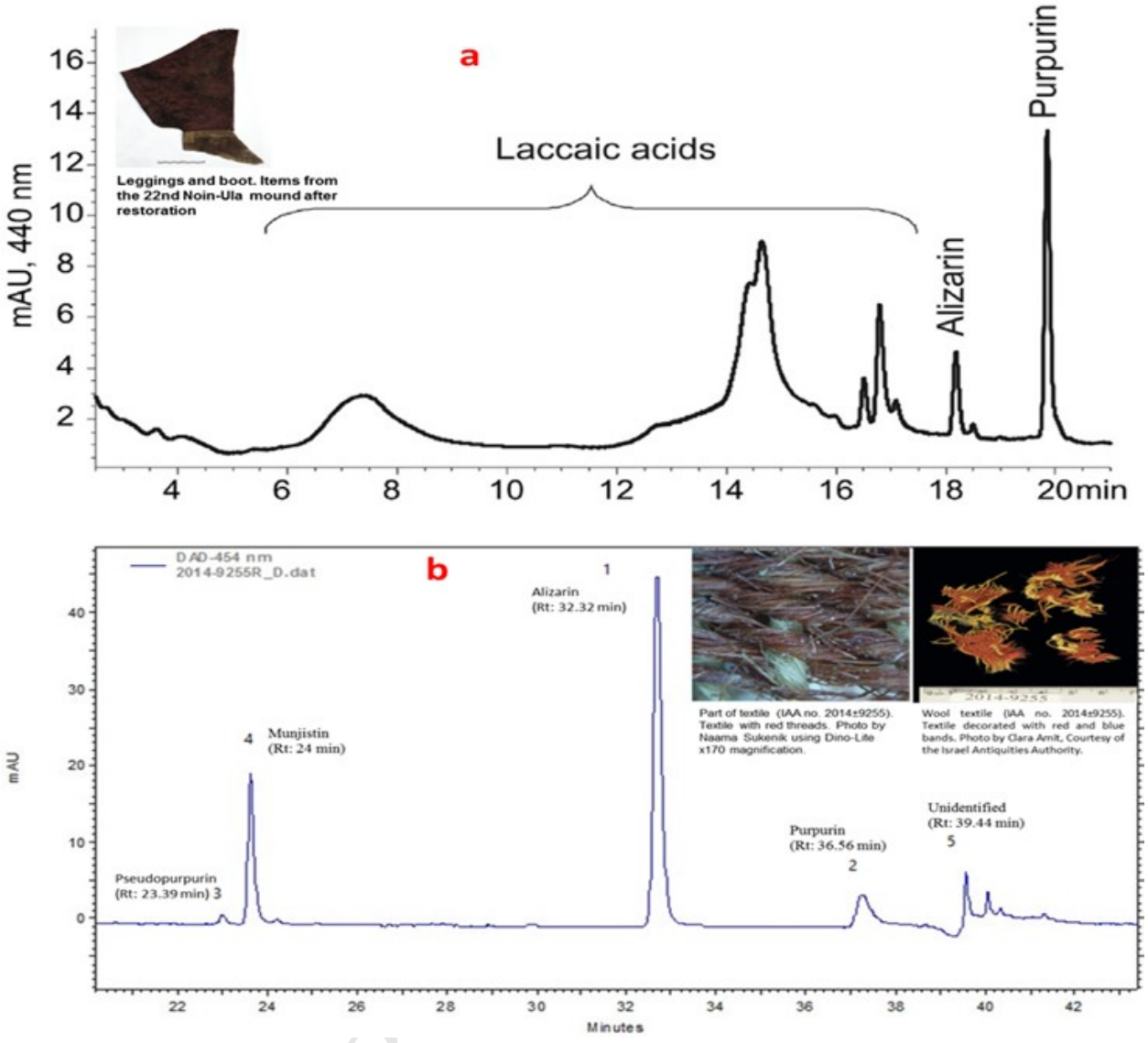

DAD data
HPLC Separation
Retention data
MS data (Full Scan)
AMDIS Filtration
IEC for the targeted molecular ion m/z
Compound present?
No
ABSENT
Yes
Confirm by t_R?
No
ABSENT
Yes
Intensity?
High
DAD confirm?
No
ABSENT
Yes
PRESENT
Moderate or Low
Other compounds from the same source are present?
No
Yes
PRESENT
Is target compound the major one from the biological source
No
ABSENT
Yes
HPLC Separation
MS data (SIM)
Confirm by t_R?
No
ABSENT
Yes
Concentrate sample
Low
Intensity?
Yes
Compound present?
No
ABSENT
MS/MS data (SRM)
HPLC Separation
Moderate or High
HPLC Separation
MS/MS data (Scan product ions or MRM)
Confirmed mass transitions?
Yes
PRESENT
No
Principal mass transitions missing?
Yes
ABSENT
No
PRESENT

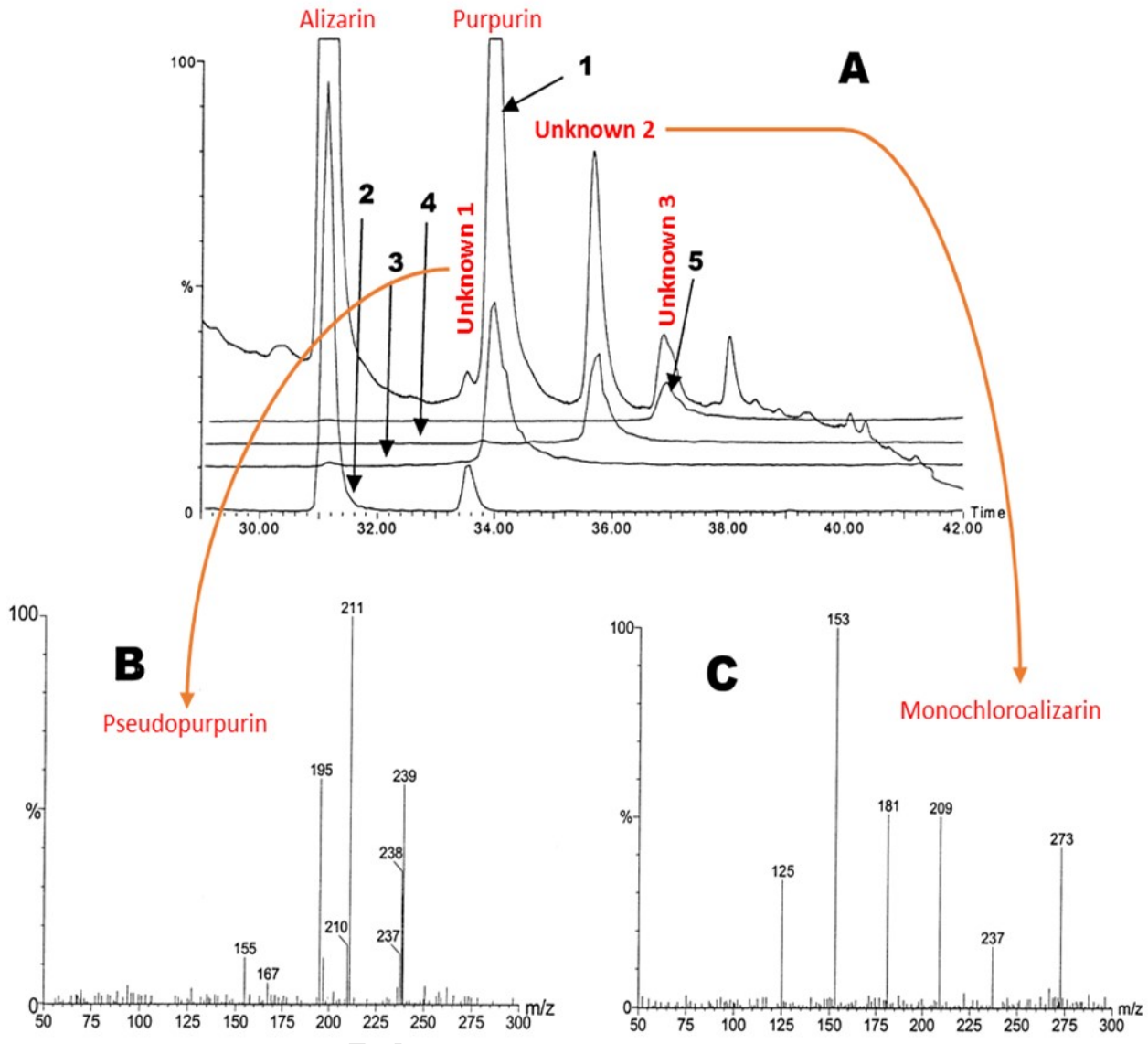
Fig. 15

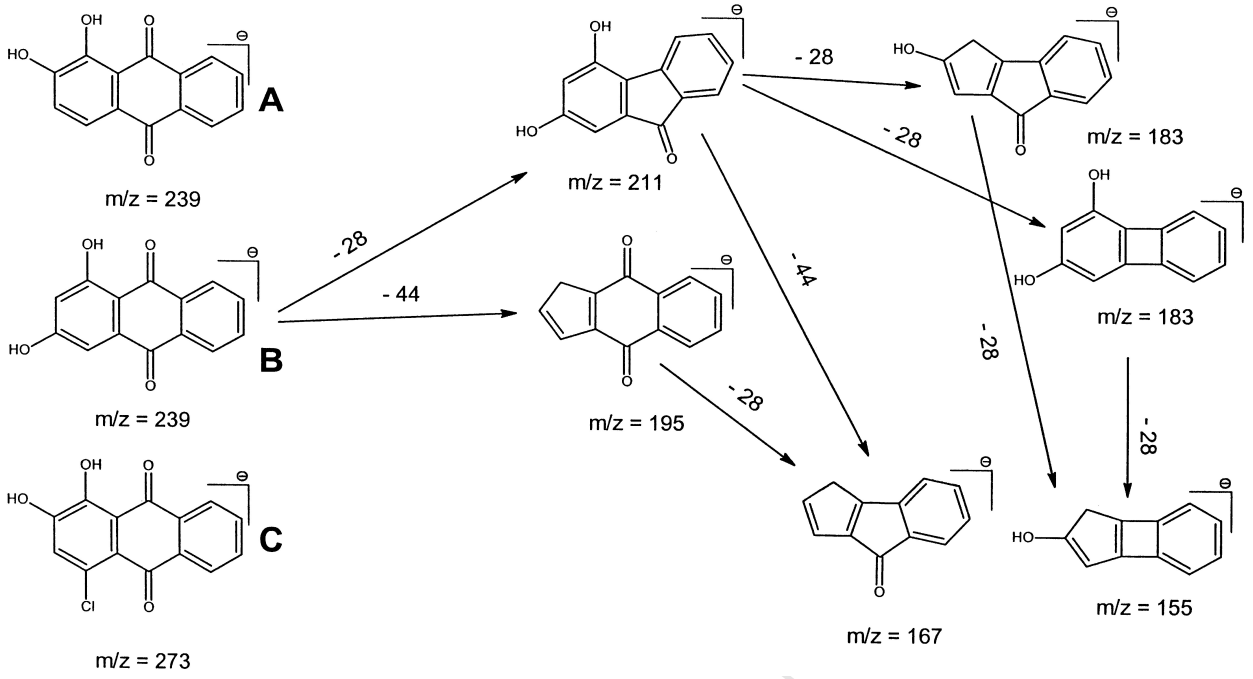

EICs: m/z 254, 268, 281, 282, 297, 298, 341
(a) 922.26.1
Rubia tinctorum
26
27
25
29
31
33
24.00
26.00
28.00
30.00
(b) 1947.ad.19
Relbunium
27
29
30
33
26.00
28.00
30.00
(c) TD2013.4.2.A
Galium tinctorium
33
34
28
27
29
30
32
24.00
26.00
28.00
30.00
Abundance
Time (minutes)

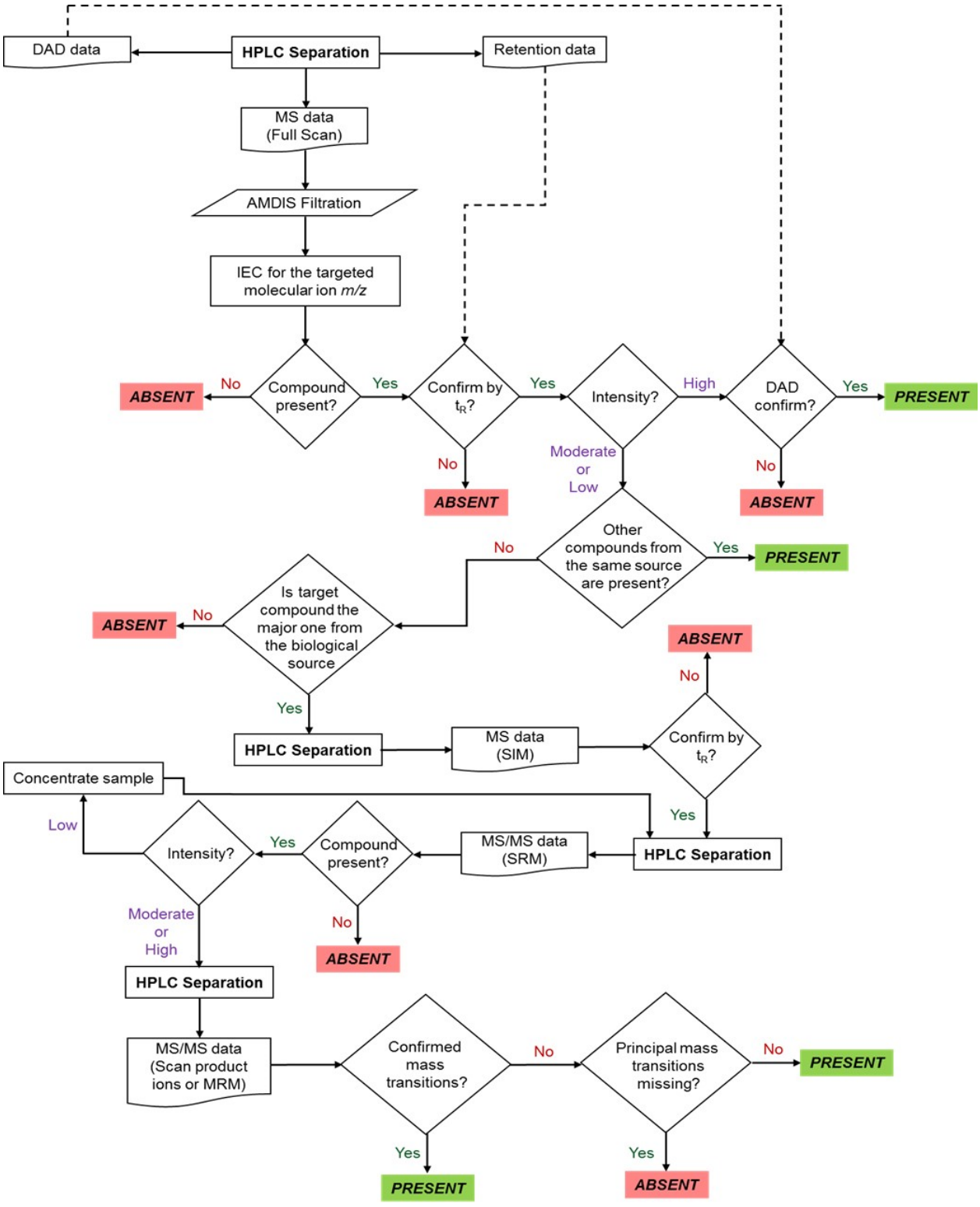

I. Shikimate Pathway
Terpenoid pathway
III.MVA Pathway
III.MEP Pathway
Chorismate/o-succinylbenzoic acid pathway
PEP + E-4-P
glucose
shikimic acid
II. TCA
chromisic acid
isochromisate synthase
isochromisic acid
α-ketoglutaric acid
+TPP
o-succinylbenzoate synthase
CO2, PEP
o-succinylbenzoic acid (OSB)
OSB:CoA ligase
CoA ester of OSB
1,4-dihydroxy-2-naphthoic acid
acetyl CoA
pyruvate
glyceraldehyde 3-P
+TPP
CO2
3-hydroxy-3-methylglutaryl CoA
1-deoxy-D-xylulose 5-P
mevalonic acid
2-C-methyl-D-erythritol 4-P
IPP isomerase
dimethylallyl phosphate
isopentyl diphosphate
Anthraquinones
1
CO2
Acetyl CoA
Malonyl CoA
CO2
octa-ß-keto-acyl chain
Polyketide pathway

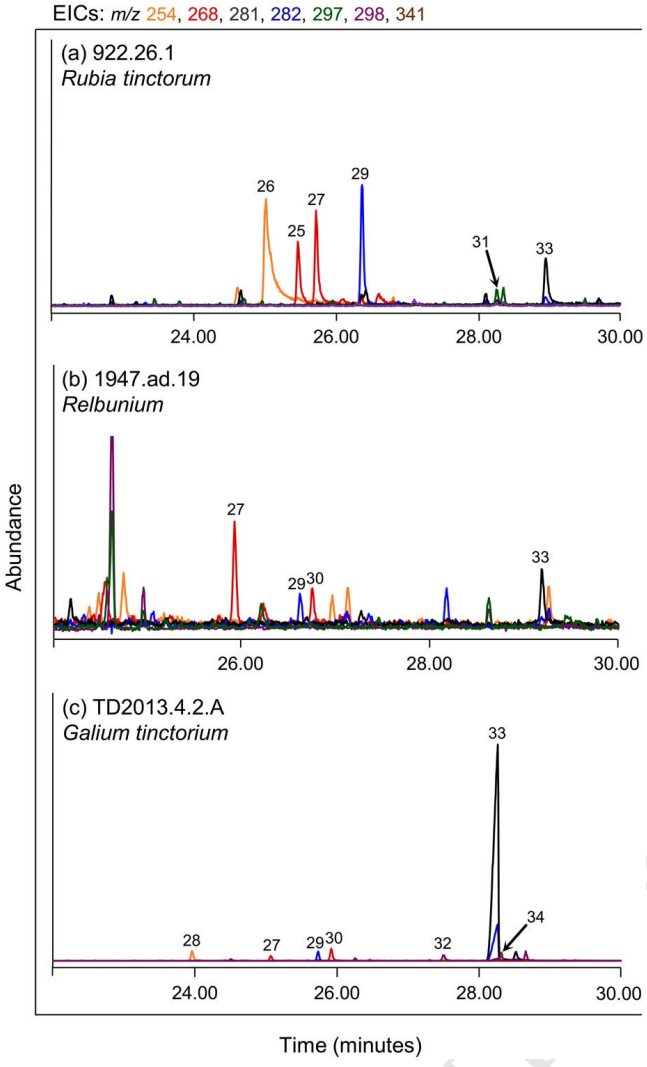

Mixture of dyestuff references (Absorbance at 275 nm)
HPLC Luna
UHPLC BEH Shield
Phosphoric acid
Formic acid
A
B
E
F
ge
al
lu
ap
in+cu
pu
in
cu
0,22
0,00
10
15
20
25
Retention time (Rt)
Mixture of insect dyes (Absorbance at 430 nm)
C
D
G
H
ca
fk
ka
dcII
0,18
Retention time (min)

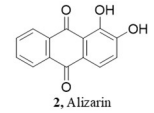

a
Laccaic acids
Alizarin
Purpurin
mAU, 440 nm
Leggings and boot. Items from the 22nd Noin-Ula mound after restoration
b
DAD-454 nm
2014-9255R_D.dat
1
Alizarin
(Rt: 32.32 min)
4
Munjistin
(Rt: 24 min)
Pseudopurpurin
(Rt: 23.39 min) 3
Purpurin
(Rt: 36.56 min)
2
Unidentified
(Rt: 39.44 min)
5
Part of textile (IAA no. 2014±9255). Textile with red threads. Photo by Naama Sukenik using Dino-Lite x170 magnification.
Wool textile (IAA no. 2014±9255). Textile decorated with red and blue bands. Photo by Clara Amit, Courtesy of the Israel Antiquities Authority.
mAU
Minutes


Alizarin
Purpurin
A
1
2
3
4
5
Unknown 1
Unknown 2
Unknown 3
100
%
0
30.00
32.00
34.00
36.00
38.00
40.00
42.00
Time
B
Pseudopurpurin
211
195
239
238
210
237
155
167
m/z
C
Monochloroalizarin
153
181
209
273
125
237
50
75
100
125
150
175
200
225
250
275
300

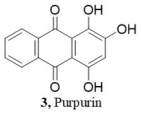



DAD data
HPLC Separation
Retention data
MS data (Full Scan)
AMDIS Filtration
IEC for the targeted molecular ion m/z
Compound present?
No
ABSENT
Yes
Confirm by t_R?
No
ABSENT
Yes
Intensity?
High
DAD confirm?
Yes
PRESENT
No
ABSENT
Moderate or Low
Other compounds from the same source are present?
No
Yes
PRESENT
Is target compound the major one from the biological source
No
ABSENT
Yes
HPLC Separation
MS data (SIM)
Confirm by t_R?
No
ABSENT
Yes
Concentrate sample
HPLC Separation
MS/MS data (SRM)
Compound present?
Yes
Intensity?
Low
Moderate or High
No
ABSENT
HPLC Separation
MS/MS data (Scan product ions or MRM)
Confirmed mass transitions?
No
Principal mass transitions missing?
No
PRESENT
Yes
PRESENT
Yes
ABSENT

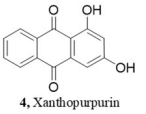




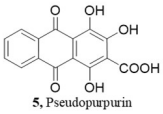
2, Alizarin



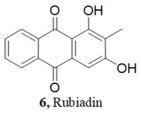
3, Purpurin



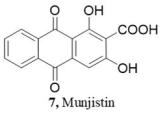
4, Xanthopurpurin



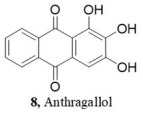
5, Pseudopurpurin



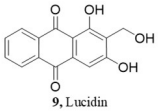
6, Rubiadin



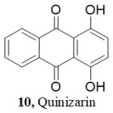
7, Munjistin



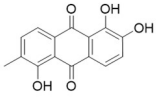
8, Anthragallol



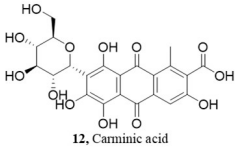
9, Lucidin



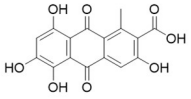
10, Quinizarin



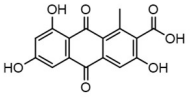
11, Morindone



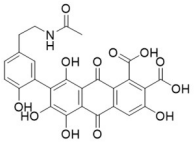
12, Carminic acid



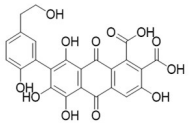
13, Kermesic acid



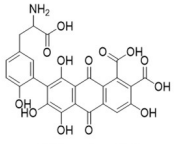
14, Flavokermesic acid (Xanthokermesic acid or Laccaic acid D)



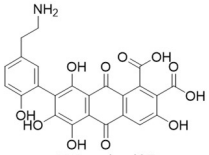
15, Laccaic acid A



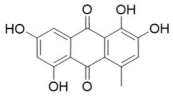
16, Laccaic acid B



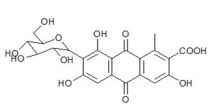
17, Laccaic acid C



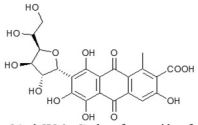
18, Laccaic acid E



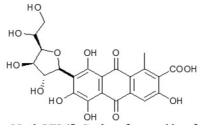
19, Erythrolaccin



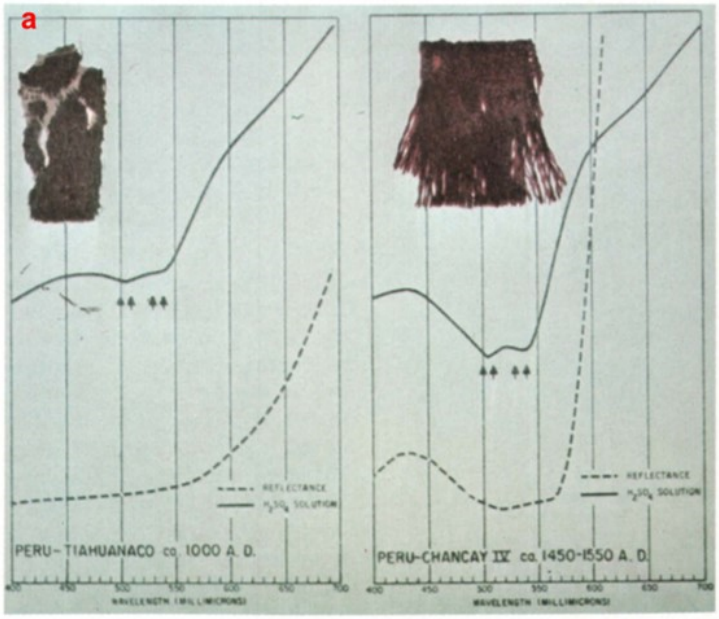
20, dcII (2-C-glucoside of flavokermesic acid)

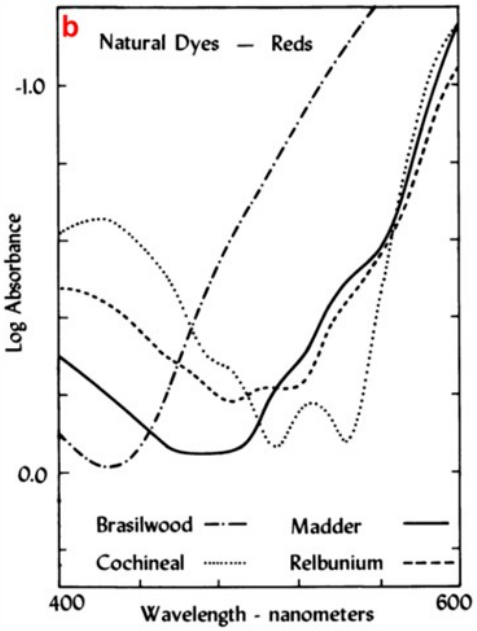


21, dcIV (α-C-glucofuranoside of kermesic acid)

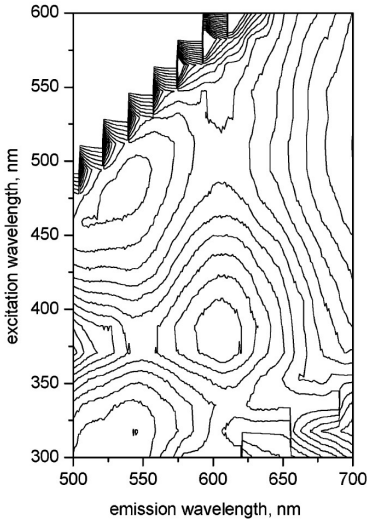


22, dcVII (β-C-glucofuranoside of kermesic acid)

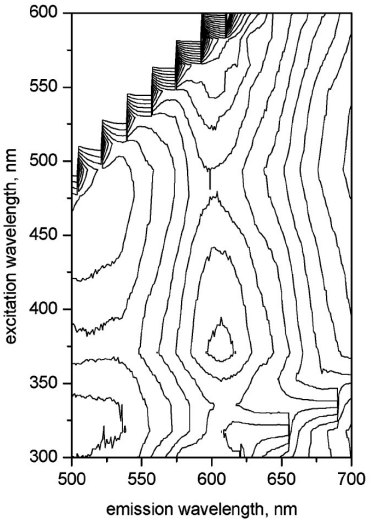




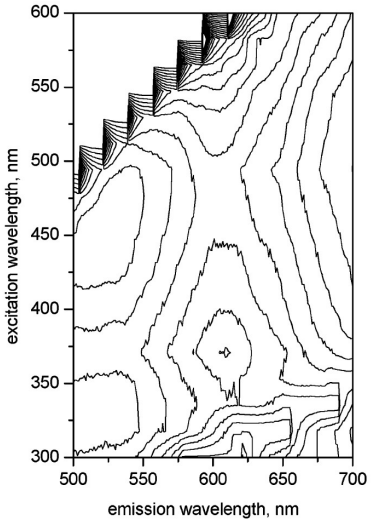
(a) *akane* red reference



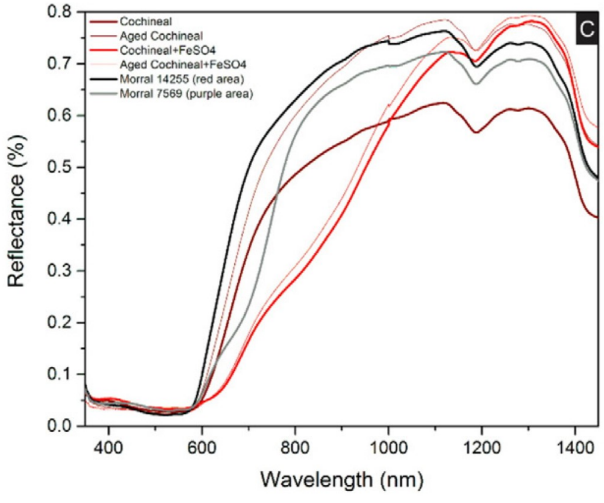
(b) red twill of the cloth lining

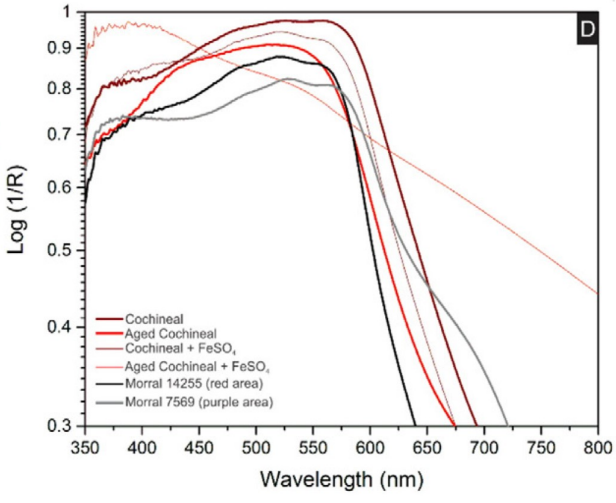


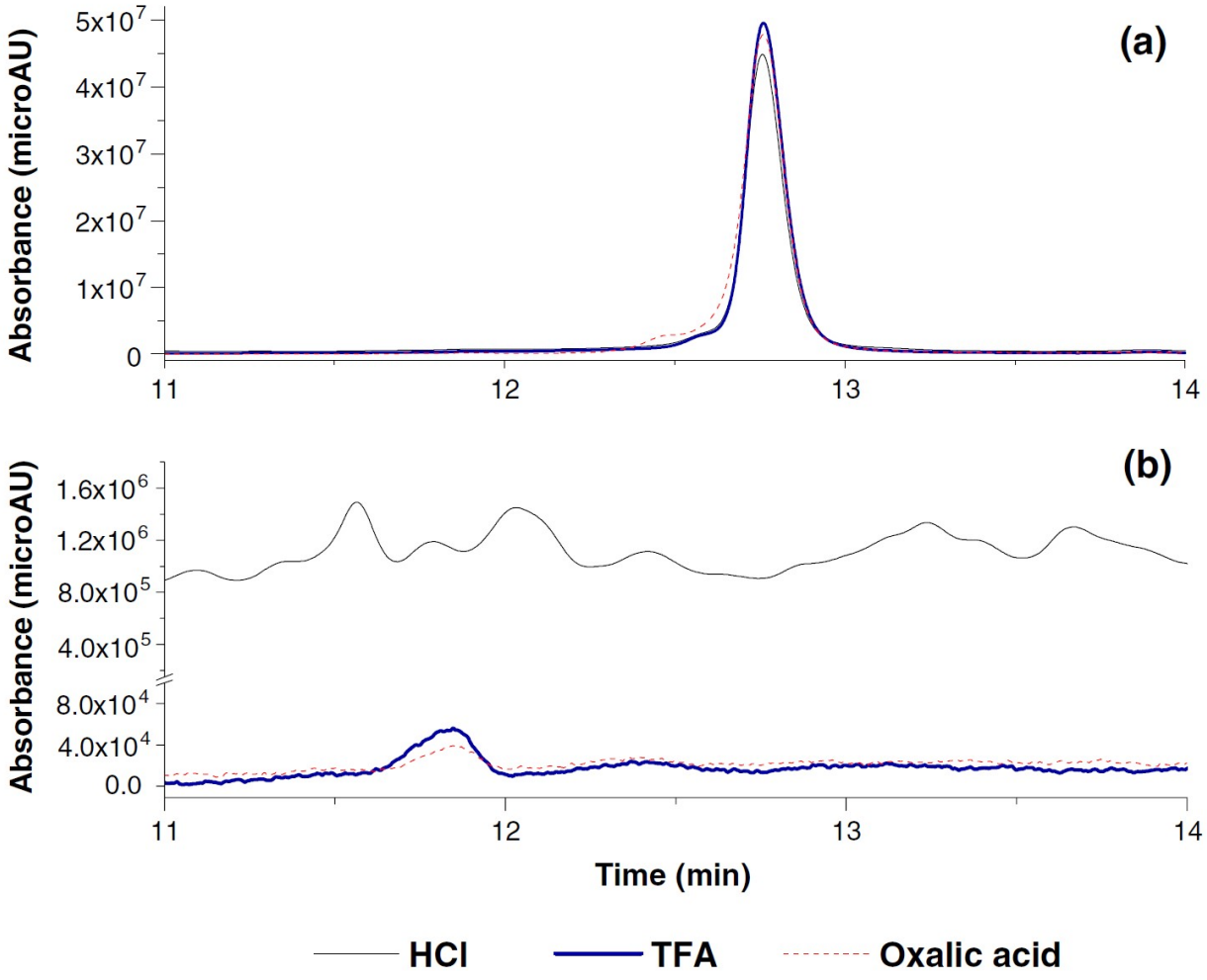
(c) red plain silk of the sleeveless coat

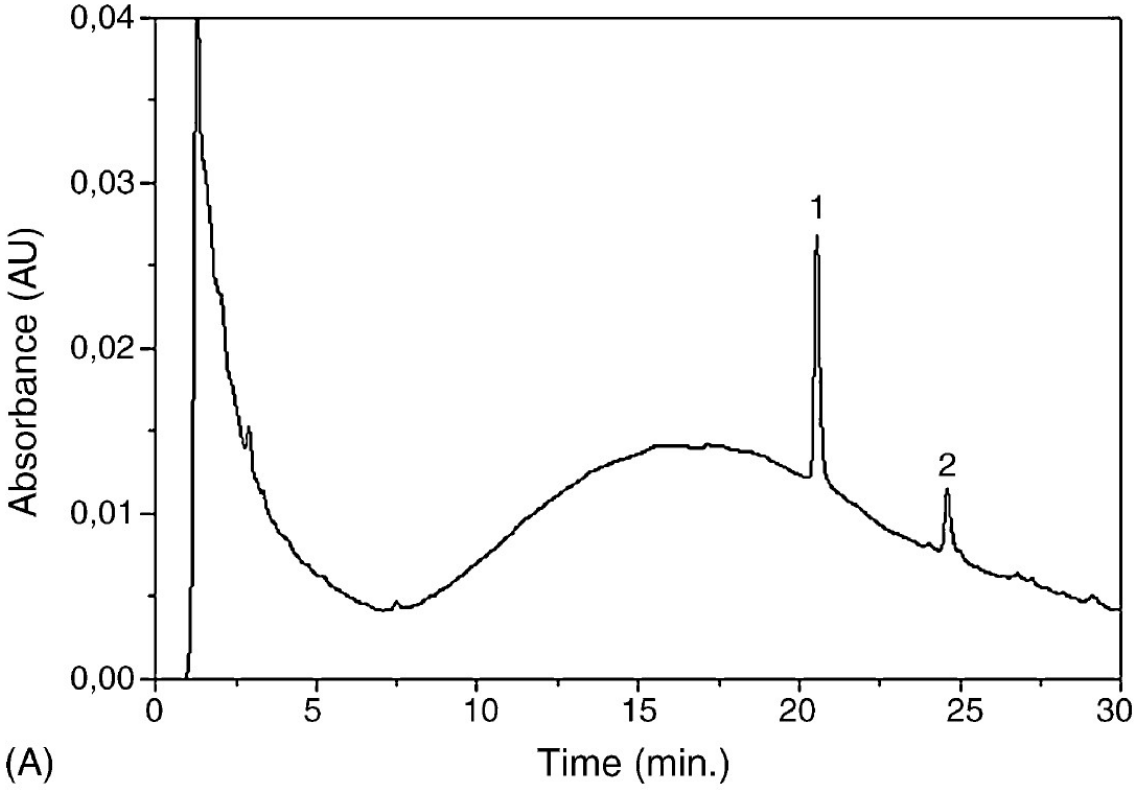



A
B

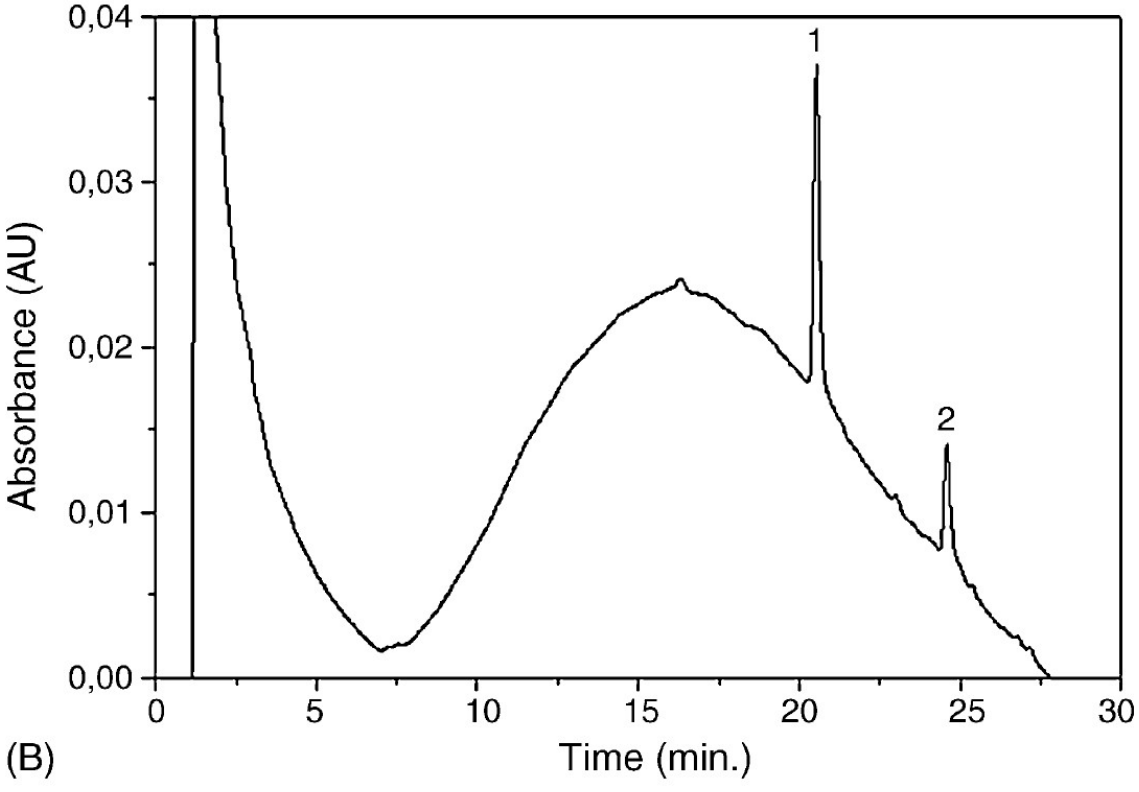

C
Cochineal
Aged Cochineal
Cochineal+FeSO4
Aged Cochineal+FeSO4
Morral 14255 (red area)
Morral 7569 (purple area)
Reflectance (%)
Wavelength (nm)


D
Cochineal
Aged Cochineal
Cochineal + FeSO4
Aged Cochineal + FeSO4
Morral 14255 (red area)
Morral 7569 (purple area)
Log (1/R)
Wavelength (nm)




0,04
0,03
0,02
0,01
0,00
Absorbance (AU)
1
2
0
5
10
15
20
25
30
Time (min.)

(A)


0,04
0,03
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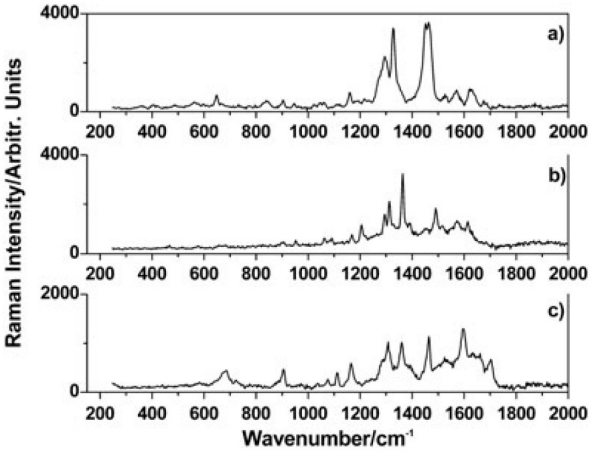
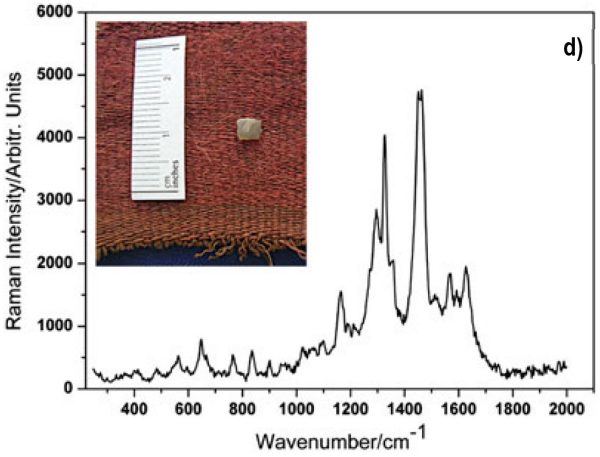
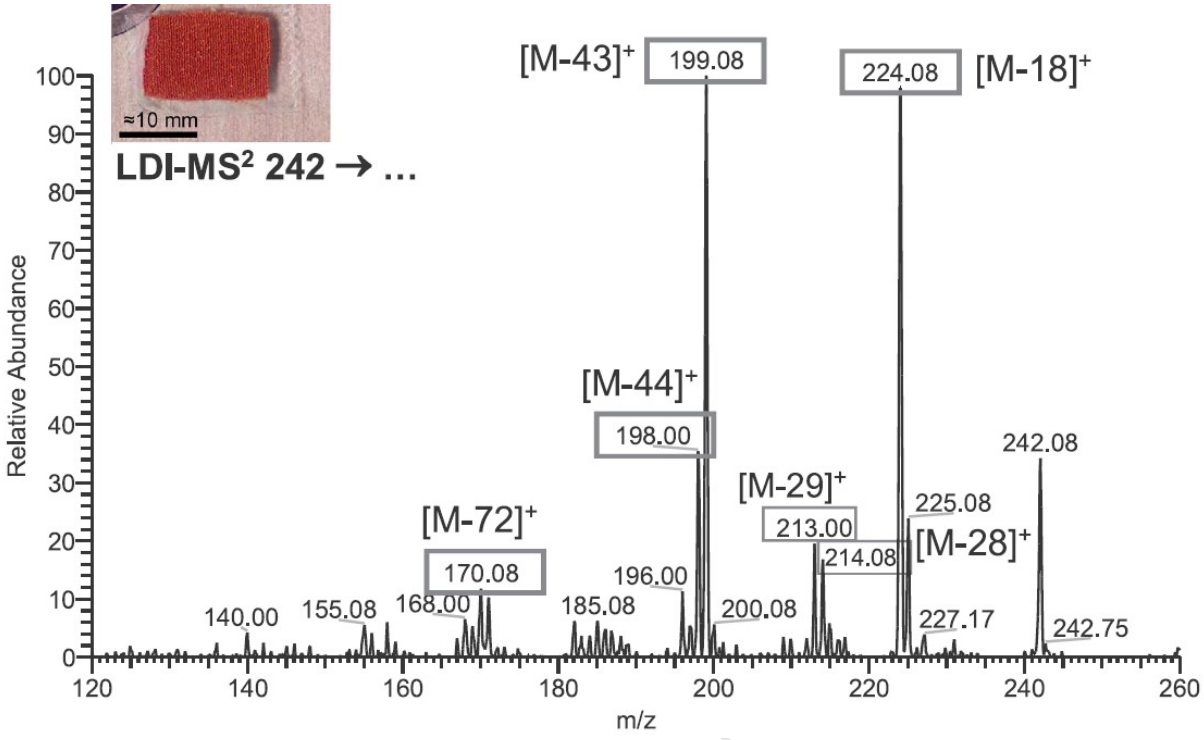
0,00
Absorbance (AU)
1
2
0
5
10
15
20
25
30
Time (min.)

(B)







Highlights

- Critical review of anthraquinone dye analysis methods for historical textiles highlighting advantages, limitations and applicability.
- Aspects relating to sampling, extraction and analysis steps are critically discussed.
- HPLC based techniques are still most widely used procedure.
- New trends include minimization of sampling requirements and development of non-destructive methods.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: