



Original article

A spectroscopic characterisation of the dyes extracted from *Iris germanica* and their identification on illuminated manuscriptsElisa Calà^a, Cristina Fornacelli^b, Federica Gulino^a, Francesca Robotti^a, Giovanni Bartolozzi^b, Marcello Picollo^b, Maurizio Aceto^{a,*}^a Università degli Studi del Piemonte Orientale, Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, piazza S. Eusebio, 5, Vercelli, 13100, Italy^b Istituto di Fisica Applicata "Nello Carrara", Consiglio Nazionale delle Ricerche (IFAC-CNR), via Madonna del piano, 10, Sesto Fiorentino, FI, 50019, Italy

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ABSTRACT

Iris flowers have been used for their dyeing properties at least since the Middle Ages and for centuries later in Europe. In particular, as regards the *Iris germanica* species, the colours that can be obtained from the petals are violet (the pure juice), dark blue (upon addition of alum) and green (upon alkalisation). The ancient technical treatises are rich with recipes describing the preparation of these dyes. Nevertheless, the dyes of *Iris germanica* have rarely, if ever, been identified on paintings. This could be due to a lack of diagnostic information, useful for the selective identification of these dyes. In fact, normally the identification of organic colourants throughout non-invasive methods is complex and generally harder than for inorganic colourants. In this study, non-invasive spectroscopic techniques, such as UV-visible diffuse reflectance spectrophotometry with optical fibres (FORS), FT-IR spectrophotometry in reflectance mode and Fibre Optic Molecular Fluorescence (FOMF), as well as micro-invasive techniques such as Surface Enhanced Raman Spectroscopy (SERS) and FT-IR spectrophotometry in transmittance mode, have been used to characterise the dyes extracted from the petals of *Iris germanica*. In the end, the methods exploited in this work have been built to move from laboratory-based information retrieved through non-invasive and micro-invasive techniques, to the *in-situ* study of manuscripts in which we identified selectively the dyes and, in particular, the iris green cited by the ancient technical treatises. Moreover, the combined application of these techniques could allow to identify its presence even in cases when the dye is mixed with verdigris or superimposed on it.

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1. Introduction

Many plant-derived colourants have attracted considerable scientific interest, not only for their aesthetic appeal but also for their functional significance. Indeed, beyond the beauty of the colours produced by their molecules, these natural dyes have long been employed in both painting and dyeing practices. The molecules responsible for plant colouration are primarily flavonoids, betalains, chlorophylls, and carotenoids. Among the flavonoids, anthocyanins – a subclass derived from phenylalanine – play a key role in producing the vivid red, purple, and blue hues typical of many vegetables, fruits, grains, and flowers. A notable example of flowers displaying such vibrant colours is found within *Iris* genus, among which the *Iris germanica* is studied in this work.

The flowers of *Iris germanica* were mentioned as a specific dye source for the first time in 1993 by Schweppe [1], but there are

several ancient texts that mention its use in miniature painting. In 12th century Theophilus Presbiter, the German monk author of the *Schedula diversarum artium* [2], in book I, recipe XXXII (*Quomodo colores in libris temperentur*) briefly cited *sucum gladioli*, a juice used to shade *viride hispanicum* (basically the mixture of copper acetates collectively known as verdigris). It can be assumed that the term “gladioli” refers to the iris flower, also known in ancient Italian as “ghiadiuolo” – a word derived from the Latin *gladius* (sword), in reference to its sword-shaped (ensiform) leaves.

The use of *zuco gladioli* (again iris juice) is recommended also by a Magister Bernardus to dilute verdigris to obtain a better green paint. The text, a treatise devoted to the technique of painting on books and datable to the 13th century, is known as *Liber colorum secundum Magistrum Bernardum* [3].

A similar recommendation is contained in the *Liber diversarum arcium*, perhaps the largest collections of technical recipes for painting, commented by Clarke in his edition of the book [4]. The collection was contained in the 14th century manuscript H 277 housed in the Bibliothèque Universitaire Historique de Médecine

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in Montpellier (France) and therefore known as the “Montpellier manuscript”. Inside the collection, the recipe §1.16.17 suggests adding *sucum gladioli* to verdigris.

In the 14th century *De arte illuminandi* by anonymous [5], a recipe in chapter X (*Del colore verde*, “On the green colour”) describes the extraction of the juice from the flowers of iris, successively used to produce a green dye upon addition of alum - $\text{Al}_2(\text{SO}_4) \cdot 3\text{K}_2\text{SO}_4 \cdot \text{H}_2\text{O}$. After this, the recipe describes in detail how to fix the dye on a *pecia* or *pezzuola*, a small piece of cloth, a practice commonly used in the Middle Ages to have a reservoir of dyes for periods in which it was not possible to extract them from plants.

Again Clarke, in his previous collection of medieval recipe books [6], lists four other manuscripts citing a green dye from iris: ms. O.8.25 (14th century) kept at Trinity College Library in Cambridge, ms. 1247 (15th century) kept at Biblioteca Riccardiana in Florence, ms. Sloane 416 or “Venetian manuscript” (15th century) kept at British Library in London, and ms. Canonici Misc. 128 (15th century) kept at Bodleian Library in Oxford.

The 15th century manuscript *Segreti per colori* (Secrets for colours) also known as “Bolognese manuscript” [7], provides several instructions involving iris-based dyes. In recipe no 92, *Affare verde chiaro per miniare optimo* (“To make a light green, excellent for miniatures”), it describes how to prepare the green dye and fix it on the *pezzuola*. Later, in recipe no 237, *A campeggiare et fare fogliami* (“To lay flat tints and make ornamental foliage”), it recommends using *la pezola de lo giglio* (“the clothlet with lily”)—iris was often referred to as *lilium*) for shading.

In the Renaissance, Felice Feliciano, an Italian artist and scholar, in his *Praecepta colorum* published in 1463, described in detail how to produce a green dye from iris flowers [8]. The recipe *A scriver di verde* (“to write in green”) says “*Prima trova del mese di marzo, osia di aprile, el fior gazuolo e quelle tre foglie pendente pista molto bene e premi fuora el sugo, e ponili dentro l'alume de roza, e poi meteli le peze de lino e bambase, prima lavade nella lescivia, e fa' che dela dita lescivia siano alquanto molle. E gietale nel dicto liquore, nel quale fai che stiano per zorni X in XII, e sugale poi al vento. E quando vorai cavare el colore verde, habii una coza con un poco de liscivio et chiaro spongato, e molto bene premila dicta peza, tanto che ne esca fuori il verde colorito, e di quello scrivi e serà bello. E se 'l dicto colore fusse troppo chiaro, strengilo un poco al sole e poi ne scrivi.*” (“At first, find in March, or April, the iris flower and mash its petals very well to extract the juice. Then add the alum and soak pieces of linen, previously washed in lye to soften them, and leave them in the solution from X to XII days and then dry them to the wind. To have green colour treat the *pezzuola* with lye and egg white and then press it”). Basically, the dye described by Feliciano is produced from *Iris germanica* by extracting the juice, adding to it a proper amount of alum which turns the colour of the juice from violet to dark blue, and then making the solution basic by adding potash to obtain a final green colour. The recipe also recommends soaking a piece of *pezzuola* in the alum solution, allowing it to serve as a reservoir for the dye.

Later on, in the 17th century *Ricette per far ogni sorte di colore* (“Recipes to do all sorts of colour”) also known as “Paduan manuscript” [7], kept in the Library of the University of Padua, the recipe nr. 86 *Per fare il verde gilio* (“To make the lily green”) recommends extracting the juice from the petals, then soak in it a clothlet together with quicklime and alum until the green colour is developed. The recipe nr. 112 *Per fare verde gilio* is similar, without citing the clothlet.

Still, in the early 17th century the Portuguese artist Felipe Nunes [9], in his treatise *Arte poetica, e da pintura e symetria, com principios da perspectiva*, described a dye called *catasol*, extracted from lily and turned to green upon addition of alum.

Ultimately, the information reported in the ancient treatises suggest that three dyes can be obtained from the petals of *Iris germanica*:

- natural violet of the pure juice extracted from the petals;
- dark blue obtained upon addition of alum;
- green obtained upon change of pH to slightly alkaline values.

In particular, the description of the green dye is coherent with the dye sometimes cited as *iris green* or *lily green* [10].

The numerous references in ancient technical treatises suggest that dyes extracted from *Iris germanica* were likely used by painters during the late Middle Ages and the Renaissance. However, to the best of our knowledge, there is scarce diagnostic evidence of the use of these dyes in painting or textile dyeing reported in the scientific literature. A first identification was reported in the late '90s by Majolino et al. [11,12] in the 13th century manuscript *Messanensis Graecus S. Salvatore* 88 kept at Biblioteca Regionale di Messina (Sicily, Italy) that was analysed by means of portable FT-IR technique. The authors identified a green dye made by iris flowers, according to two main bands at 1124 cm^{-1} and 1596 cm^{-1} attributed to the juice, and a band at 1040 cm^{-1} attributed to alum, with a good correspondence to the recipe by Felice Feliciano. Recently, Rönnerstam et al. [13] analysed a colour chart dated to 1680, titled *Nomenclatura et species Colorum Miniatae Picturae*, Thet är: Förteckning och Proff på Miniatur Färgår. Here, a dark greenish sample, labelled in Latin, French, and Swedish as *Succus foliorum gladioli/le verd d'Iris/Iris grönt*, exhibited a reflectance minimum approximately at 605 nm when examined using UV-visible diffuse reflectance spectrophotometry with optical fibres (FORS), while no inorganic components were detected by XRF analysis. Based on these results, the authors suggested that the material used for the green dye was consistent with iris green. According to references in ancient treatises, iris dyes are expected to be found mainly on illuminated manuscripts, which are both highly precious and extremely fragile. These requisites usually require a non-invasive approach to preserve the integrity of materials, with non-negligible limitations on dye identification. Therefore, as a consequence of the limited availability of diagnostic information, the identification of iris-based dyes is so far still challenging.

2. Research aims

Most spectroscopic studies concerning Iris-derived compounds, anthocyanins or related plant colourants have been mainly carried out in botanical, phytochemical or food-science contexts, generally on extracts or solutions rather than on solid paint layers applied to historical substrates [14–17]. As a consequence, these data cannot always be directly transferred to the interpretation of historical materials, where the spectral response may be affected by the substrate, the preparation method, the presence of binding media, ageing phenomena and possible mixtures or superimpositions with other pigments.

During the Middle Ages, iris green has been introduced as a popular alternative to other green pigments, especially in manuscript illumination, where potentially reactive copper-based greens, such as verdigris, could raise concerns due to their interaction with delicate organic substrates. In this context, the present study was designed as a broad and comprehensive analytical screening, mainly focused on non-invasive techniques, aimed at assessing both the potential and the limitations of different spectroscopic methods for the study of *Iris germanica* dyes applied on parchment. The aim was therefore twofold: to identify useful diagnostic features, where possible, and to clarify what type of information can realistically be obtained from each technique, avoiding overinterpretation of the analytical results. Such an approach is intended to provide heritage scientists and conservators with a

reference framework for choosing the most appropriate analytical strategy according to the specific constraints of each case study, especially when sampling is not allowed or only very limited micro-sampling is possible.

To improve the current knowledge on the dyes extractable from *Iris germanica*, as well as their possible identification in manuscripts and more generally in historical artworks, a spectroscopic study combining non-invasive and micro-invasive techniques was carried out. The potentialities of the use of non-invasive analytical methods, such as UV-visible diffuse reflectance spectrophotometry with optical fibres (FORS), Fourier Transform Infrared spectroscopy (FT-IR) and fibre optic molecular fluorimetry (FOMF), was investigated on reference mock-ups prepared on parchment. The experimental conditions were therefore optimised for the analysis of solid samples rather than solutions, with the aim of identifying spectral features useful for detecting iris-based dyes on manuscript materials.

In addition, Surface Enhanced Raman Spectroscopy (SERS) has been used to improve the identification in cases where micro samples were available.

3. Materials and methods

3.1. Extraction of dyes from *Iris germanica* flowers

Dyes were prepared following a recipe cited by Ziggotti [18] that recalls closely the recipe of Felice Feliciano. Flowers of *Iris germanica* were collected in full bloom (Figure S1) and then stored in freezer. For the extraction, 10 g of frozen petals were previously rinsed with ultrapure water, then boiled in 100 g of water under stirring for 10 min (Figure S1). The solution, with a pH of approximately 6, quickly turned dark violet, indicating successful extraction of the dyes, and was then separated from the petals by filtration. For reference, this extract will hereafter be referred to as *iris violet*.

3.2. Preparation of mock-up samples

The addition of 0.5 g of alum to the violet solution caused it to turn dark blue under stirring for five minutes. This mixture is hereafter referred to as *iris blue*. Finally, KOH was added to adjust the pH to 10, producing a green solution, hereafter referred to as *iris green*.

The solutions were purified by filtration through Whatman No. 1 filter paper. The resulting solutions were kept in refrigerator at 4 °C in order to stop any fermentation process. In these conditions, the dye solutions were found to be stable for several months.

After purification, small drops of each solution were applied to parchment and allowed to dry. The process was repeated several times, until an intense colour was achieved for each dye. Figure S1 shows the resulting colours of the three dyes on parchment.

Mixtures of iris green and verdigris were prepared by mixing a few drops of iris green, 10 mg of verdigris and a drop of gum Arabic solution as binder. The gum Arabic solution was prepared according to the recipe described by Ziggotti [18], that is 1 part of gum Arabic and 1 part of distilled water; the solution was ready for use after 3 days. For overpaints of iris green on verdigris, a paint of verdigris was firstly prepared by mixing 10 mg of verdigris with a drop of gum Arabic solution and applying it to parchment, then, after drying, a few drops of iris green were applied on top of the verdigris paint.

3.3. Fibre optic reflectance spectrophotometry (FORS)

FORS analysis was performed with an Avantes (Apeldoorn, The Netherlands) AvaSpec-ULS2048XL-USB2 model spectrophotometer

and an AvaLight-HAL-S-IND tungsten halogen light source; detector and light source were connected with fibre optic cables to an Avantes FCR-7UV200–2–1.5 × 100 probe. In this configuration, both the incident and detecting angles were 45° from the surface normal, in order not to include specular reflectance. The spectral range of the detector was 200–1160 nm; the overall operational range of the device (combination of lamp + detector) was 375–1100 nm. Depending on the features of the monochromator (slit width 50 µm, grating of UA type with 300 lines/mm) and of the detector (2048 pixels), the best spectra resolution was 2.4 nm calculated as FWHM. Diffuse reflectance spectra of the samples were referenced against the WS-2 reference tile provided by Avantes and guaranteed to be reflective at 98% or more in the spectral range investigated. The investigated area on the sample had a 1 mm diameter. The probe was held by hand and in all measurements the distance between probe and sample was kept constant to 0 mm. The instrumental parameters were as follows: 10 ms integration time, 100 scans for a total acquisition time of 1.0 s for each spectrum. The whole system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 11™.

3.4. FT-IR spectroscopy

FT-IR analysis was carried out with a portable Bruker Optics Inc. (Billerica, MA, USA) spectrometer model ALPHA II, supplied with the external reflectance and transmittance modules. The spectrometer was equipped with a SiC globar type source (silicon carbide with emission comparable to that of a black body with a temperature of about 1000 K) and a DTGS pyroelectric detector (deuterated triglycine sulphate). For each spectrum, 256 scans were acquired in the spectral range 7500–375 cm^{−1} with a resolution of 4 cm^{−1}. Data were processed using OPUS 9.0.6 and EZ OMNIC 7.3 software. Background spectra were acquired on a gold mirror for reflectance measurements and on pure KBr pellets for transmission mode.

The iris dye extracts were analysed in transmission mode, using KBr pellets to maximise spectral transparency and allow direct comparison with reference spectra. On the contrary, the iris-based paint layers applied on parchment were investigated in external reflectance mode, a non-invasive configuration suited for in situ analysis of painted or coated surfaces.

It should be noted that FTIR in reflectance mode is affected by distortions in the mid infra-red region, caused by anomalous dispersions due to variations in the refractive index across an absorption band [19]. To minimise these effects, Kramers-Kronig algorithm (KK) - included in the OMNIC software package - was applied to the reflectance spectra to obtain pseudo-absorbance spectra. The use of the KK algorithm compensates for phase distortions associated with specular reflection, allowing reliable comparison with transmission mode and database reference spectra [20].

3.5. Fibre optic molecular fluorescence (FOMF)

FOMF measurements were carried out using an Avantes AvaLight-HPLED-385 LED source as excitation source, a Semrock (Rochester, NY) 442 nm EdgeBasic™ long-pass edge filter accommodated inside an Avantes FH-INLINE filter holder to selectively remove the Rayleigh line, and an Avante AvaSpec-ULS2048XL-USB2 spectrophotometer to collect the fluorescence emission light. Both detector and LED source were connected with fibre optic cables to an Avantes FCR-7UV200–2–1.5 × 100 probe. The spectral features were the same as FORS analysis. The instrumental parameters were as follows: 0.5 s integration time, 10 scans for a total acquisition time of 5 s for each spectrum. The whole system was managed by means of AvaSoft v. 8 dedicated software, running under Windows 11™. The edge filter with cutoff at 442 nm was used in order to remove the Rayleigh emission provoked by the elastic scattering of

the 385 nm LED source, therefore allowing to use higher acquisition times.

3.6. Surface enhanced Raman spectroscopy (SERS)

Raman analysis of iris dyes could not be performed using conventional modes. Measurements on mock-ups with 532, 633 and 785 nm lasers all yielded spectra lacking useful features due to high levels of fluorescence. Consequently, the SERS method was employed, as is common practice for the analysis of dyes.

SER spectra were collected with a high-resolution dispersive Horiba (Villeneuve d'Ascq, France) LabRAM HR Evolution model spectrometer coupled with a confocal microscope. The instrument was equipped with 532, 633 and 785 nm excitation lasers, a 1800 lines/mm dispersive grating, an 800 mm focal length achromatic flat field monochromator and a multichannel air-cooled CCD detector. The spectral resolution was 2 cm^{-1} . Spectra were taken with long working distance 50x and 80x objectives. The 532 nm excitation laser resulted to give the best results, so its use was optimised. Laser power at the sample was $< 1\text{ mW}$. Exposure time was 1–10 s according to needs (3 accumulations). Good and reproducible spectra were obtained in this way. The system was managed with LabSpec 6 software running under Windows 11™.

SERS analysis was performed by means of Ag nanoparticles colloidal pastes (AgNP), according to the procedure developed by Idone et al. [21], based on the method developed by Brosseau et al. [22]. A drop of dye solution was poured on a glass and allowed to dry; then 1 μL of AgNP was poured on it and allowed to dry before exposing it to the laser beam.

3.7. X-ray fluorescence spectrometry (XRF)

XRF measurements were performed with an EDXRF Thermo (Waltham, MA, USA) NITON spectrometer XL3T-900 GOLDD model, equipped with a Ag tube (max. 50 kV, 100 μA , 2 W), a large area SDD detector and an energy resolution of about 136 eV at 5.9 keV. The analysed spot had an average diameter of 3 or 8 mm and was focused by a CCD camera, with a working distance of 2 mm. The total time of analysis was 60 s. The instrument was held in position with a moving stage allowing micrometric shifts, in order to reach the desired probe-to-sample distance; the stage was laid on a tripod.

3.8. Manuscripts analysed

The list of illuminated manuscripts in which iris dyes were identified is reported in Table 1, with information concerning their location and historical period. All the manuscripts analysed are on parchment and in good conservation conditions. The identification of iris green was obtained in all instances by means of FORS analysis, according to the spectral features that will be presented in paragraph 4.1. Representative FORS spectra taken from the manuscripts, together with the images of the areas analysed, can be seen in Table S1.

4. Results and discussion

Before proceeding, it is important to recall the chemical composition of the dyes extracted from the petals of *Iris germanica*. Thompson [23] in 1956 suggested that the primary colouring matter of iris green could be chlorophyll. According to Schweppe [1], instead, the flavonoid mangiferin or 2- β -D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one was the main colourant. While not addressing the green dye specifically, Ashtakala and Forward [24] proposed in 1975 that the violet-blue colour of *Iris*

germanica petals arises from the anthocyanin delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (delphinin), likely in combination with mangiferin through copigmentation effects. Mizuno and Iwashina [25] confirmed that the violet colour is due to copigmentation of delphinin (also called violanin) with other flavonoids, among which mangiferin and swertisin 2''-O-arabinoside. Therefore, in the following discussion the characterisation of the dyes is to be referred mainly to the presence of anthocyanins that turned from violet to blue to green upon addition of chemical reagents.

A recent study by Bahreini et al. [26] further characterised the pigments of *Iris germanica*, confirming that its colour arises from a mixture of anthocyanins—notably cyanidin, delphinidin, petunidin, malvidin, and pelargonidin—together with their glycosylated derivatives. The same authors verified also the bathochromic shifts, and therefore the change in visual colour, occurring after metal complexation by means of Al^{3+} and Cu^{2+} ions. The first one is of particular interest because, as cited previously, alum was used in nearly all the ancient recipes to change the colour of the natural iris juice from violet to blue and eventually to green.

After replicating the recipe described by Felice Feliciano, we characterised the spectral features of the three dyes that can be obtained from *Iris germanica*: (1) iris violet from the pure juice, (2) dark iris blue upon addition of alum and (3) iris green upon alka-lisation.

4.1. Reflectance spectroscopy (FORS)

The absorption properties of the dyes extracted from *Iris germanica* have been already described before. Mizuno and Iwashina [25], analysing the violet aqueous extract at natural pH indicated a main absorption maximum at ca. 580 nm with two shoulders at 544 and 636 nm. Bahreini et al. [26] found an absorption maximum at 538 nm, which underwent a bathochromic shift at 584 nm upon addition of Al^{3+} , explained by the authors with the fact that the metal coordination stabilises the quinoidal base structures of the anthocyanins. However, in both cases, the reported spectral data were acquired in absorption mode in solution, which is not necessarily equivalent to spectra obtained in reflectance mode from solid samples. Moreover, the absorption measurements are typically not feasible when analysing miniature paintings, as this approach is invasive and destructive. Therefore, in this section we present the spectral information acquired in reflectance mode, the only technique suitable for non-invasive analysis, with the aim of identifying diagnostic spectral features useful for detecting these dyes on manuscripts. The absorption band, i.e. the reflectance minimum, will be the diagnostic spectral feature useful for the identification of the iris dyes cited in the ancient treatises, while the reflection band is not a reliable spectral feature as it can shift according to the concentration of the dyes or to mixture with other colourants [27,28].

4.1.1. Iris violet

Violet is the natural colour of the flowers and the colour of the pure juice obtained by pressing them. Its reflectance spectrum, shown in Fig. 1 in Log(1/R) coordinates (solid line), is dominated by an absorption band centred around 590 nm with well-defined shoulders at 549 and 629 nm, and by a reflectance band centred around 462 nm with a shoulder beyond 770 nm. Considering the pH of the juice, these features should correspond to the quinoidal anhydrobase form of the anthocyanins present.

The spectral features are compatible with those reported by Mizuno and Iwashina [25], but are somewhat different from those reported by Bahreini et al. [26], possibly due to the difference between the measurement in solid and in solution state.

Table 1

List of manuscripts in which iris green has been identified.

Manuscript	Library	Date
<i>Libro d'Ore</i> , ms. 449/M	Torino, Museo Civico di Arte Antica	1450–1460
<i>Ercole Senofontio</i> , ms. B.P. 1099	Padova, Biblioteca Civica	1463
<i>Memorabilia</i> , ms. Reg. Lat. 1388	Città del Vaticano, Biblioteca Apostolica Vaticana	1463
<i>Collectio antiquitatum</i> , ms. alfa.I.5.15	Modena, Biblioteca Estense Universitaria	1465
<i>Epigrammata</i> , ms. Vat. Lat. 3616	Città del Vaticano, Biblioteca Apostolica Vaticana	1478/1479
<i>Libro d'Ore detto di Ippolita Maria Sforza</i> , ms. A617	Rivoli, collezione Cerruti	1480/1488
<i>Offiziolo Durazzo</i> , ms. Arm. 1	Genova, Biblioteca Civica Berio	1502/1503
<i>Consolato dell'arte della seta. Matricole</i>	Napoli, Archivio di Stato	1514–1557
<i>Commissione dogale del Doge Andrea Gritti</i>	Torino, Archivio di Stato	1531
<i>Graduale</i> , choral V	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral XI	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral XII	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral XIII	Alessandria, Museo Civico	1568–1585
<i>Antifonario</i> , choral XXIV	Alessandria, Museo Civico	1568–1585
<i>Antifonario</i> , choral XXVII	Alessandria, Museo Civico	1568–1585
<i>Antifonario</i> , choral XXXIII	Alessandria, Museo Civico	1568–1585
<i>Innario</i> , choral XXXVI	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral Q (XLII)	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral H (XLIII)	Alessandria, Museo Civico	1568–1585
<i>Graduale</i> , choral R (XLIV)	Alessandria, Museo Civico	1568–1585

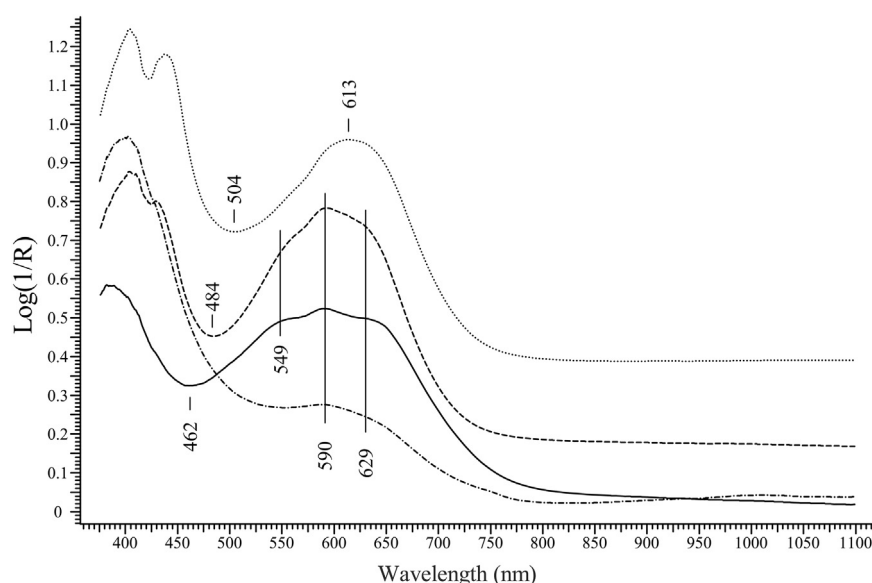


Fig. 1. FORS spectra in $\text{Log}(1/R)$ coordinates of iris violet obtained from flowers of *Iris germanica* (solid line), iris blue obtained by addition of alum (dashed line), iris green obtained by addition of KOH (dotted line) and iris violet taken to pH 10 without alum (dashed-dotted line). Spectra are offset for clarity on Y axis.

4.1.2. Iris blue

The iris blue, obtained by addition of alum, has spectral features similar to the violet (Fig. 1, dashed line). The reflectance maximum centred at 484 nm accounts for its blue colour, while the absorption band is again centred at 590 nm with less pronounced shoulders at 549 and 629 nm. The addition of Al^{3+} ions, therefore, seems not to change the absorption properties of the dye. Even increasing the alum concentration up to 50-fold did not affect the absorption spectra (data not shown). An interesting feature is that no precipitation of Al hydroxide occurs, as it is usual in the preparation of lakes (see [18]), even if the pH is brought up to 10 (see later), which suggests a strong interaction between Al^{3+} and anthocyanins.

4.1.3. Iris green

The spectrum of iris green (Fig. 1, dotted line) shows an absorption band centred at 613 nm. The reflection band is centred around 504 nm, accounting for the green colour. This behaviour, however, simply reflects the typical change of anthocyanin solutions from violet-blue to green as the pH shifts from acidic to neutral and

then to alkaline [29]. In this process, the flavylium cation is turned to the chalcone form, resulting in a significant bathochromic shift of the absorption band.

Following this spectral evidence, the influence of pH and Al^{3+} coordination on the spectral features of the dyes was further investigated. A violet extract was adjusted to pH 10 using KOH without prior addition of alum. As expected, the solution turned green, as indicated by the reflectance maximum in the FORS spectrum (Fig. 1, dotted-dashed line). However, the absorption behaviour remained essentially the same as that observed in the presence of alum.

Based on these results, the role of alum in the preparation of iris green - described in the ancient treatises - was reconsidered. Rather than modifying the colour, alum may have been used as a mordant when soaking small clothlets - the aforementioned *pez-zuole* - in the juice solution. In this process, alum and the Al^{3+} ion acted to ensure the anchoring of dye molecules to the textile substrate, acting as a mordant and forming a chemical bridge between the fibre and the dye. This common medieval practice allowed the dye to be stored and used even when fresh plant material was not

available. The juice solution could then be easily reconstructed by treating a piece of clothlet in alkaline media.

Another possible explanation is that artisans were aware that alum – or, more precisely, the Al^{3+} ion – could stabilise the colour of anthocyanins, as demonstrated by several studies reporting the formation of coordination complexes [30].

4.1.4. Role of pH in colour variation

To elucidate the influence of pH and alum on the colour variation of the iris dyes, the violet and blue extracts were subjected to controlled pH adjustments. Small volumes of 2 M KOH were added to each dye solution to modify the pH without significantly altering the concentration. The procedure was like this: firstly, the desired pH value was set with KOH, then a drop of the solution was withdrawn and deposited on parchment; after drying, all the reflectance spectra were collected from the drops. For the iris violet extract, the pH was varied from 5.1 to 10.4, while for iris blue extract, the range was 3.0–10.1. The lower initial pH observed for iris blue resulted from the acidic conditions produced by the hydrolysis of alum. The colour changes induced by pH variation were evaluated using reflectance spectroscopy, and the results are presented in Fig. 1. These reflectance plots enable a clearer visualisation of the chromatic shifts occurring across the tested pH ranges. As it can be seen (Fig. 2, top spectrum), the spectral features typical of iris violet, i.e. a main absorption band at 590 nm with two minor bands at 549 and 629 nm, are kept almost unaltered in the pH range 5.1–10.4, while a bathochromic shift occurs for the reflectance maximum, that moves from ca. 470 nm to 520 nm, accounting for the change from violet to green.

The behaviour of iris blue (Fig. 2, bottom spectrum) is more significant from the diagnostic point of view, because, according to the ancient recipes, alum was always added to the juice, after which the pH was increased to obtain the green colour. This behaviour is different from iris violet: as the pH changes from acid to alkaline values, the main absorption band at 590 nm becomes less perceivable, while a second band at 613 nm, typical of iris green, becomes dominant. The shift in the reflectance maximum is narrow, but it is enough to change the colour from blue to green, which seems to occur between pH 8 and pH 9.

According to the results described below, the identification of iris green in historical paintings is strongly influenced by the pH conditions employed during the preparation of the dye. The principal spectral feature associated with iris green is an absorption band at 613 nm, which becomes prominent at pH values above 8. Additionally, the presence of a band at 590 nm, with possible shoulders at approximately 549 and 629 nm, may also serve as diagnostic indicators, particularly in cases where the dye was prepared in moderately alkaline conditions and already exhibited a green hue.

4.1.5. Mixtures of verdigris and iris green

It is of particular interest to assess the possibility of identifying iris green when mixed with verdigris, as this specific combination is mentioned in several medieval treatises [2–4]. Given that the absorption features of individual components are generally discernible in the reflectance spectrum of a mixture, the spectrum of a verdigris/iris green mixture should display the principal spectral characteristics of both constituents, provided that the proportions are not heavily biased toward one component. The reflectance spectrum of verdigris is characterised by a wide absorption band centred at ca. 720 nm [28] (Fig. 1, solid line). The spectrum obtained from the verdigris/iris green mixture (Fig. 3, dotted line) shows that the spectral features of verdigris are modified by the presence of iris green: as the concentration of iris green increases, a second absorption band emerges at shorter wavelengths, corresponding to the dye. However, it should be noted

that the absorption band becomes evident only when a substantial amount of iris green is present. When iris green is applied on top of verdigris (Fig. 3, dashed-dotted line), the spectral features are similar to those of the mixture with a significant amount of iris green, due to the fact that the dye has a very low hiding power, therefore the absorption properties of verdigris are clearly visible.

4.2. FT-IR spectroscopy

So far, few studies have reported detailed spectroscopic data on anthocyanins, and comprehensive investigations that accurately correlate band patterns with molecular structure are still lacking [31]. Nevertheless, previous works on different *Iris* species allowed identifying and isolating multiple flavonoids, including anthocyanins [32].

Anthocyanins are polyphenolic chromophores with aromatic rings linked to oxygen-containing heterocyclic systems, often further substituted or connected to additional aromatic units through C–C bonds [33]. The purple-to-blue colour of iris flowers is predominately attributed to acylated glycosides of delphinidin, malvidin and petunidin, while reddish to purple hue are associated to cyanidin and peonidin derivatives [34].

Considering both the intrinsic spectral complexity of anthocyanins and the significant influence of environmental and matrix-related factors, the identification of characteristic FTIR bands must be approached with caution. The following section therefore summarises the main absorption features commonly reported for anthocyanin-based systems, highlighting their variability and the potential overlap with contributions from other components typically present in complex matrices.

A characteristic band in the $\sim 1655\text{--}1630\text{ cm}^{-1}$ region is commonly observed in anthocyanins, generally attributed to aromatic C=C skeletal vibrations of the conjugated flavonoid system, with possible contributions from C=O stretching depending on the molecular form (e.g. flavylium, quinoidal base or chalcone) and surrounding environment (e.g., water or protein substrates) [31,35,36]. The $1700\text{--}1600\text{ cm}^{-1}$ region is strongly influenced by H–O–H bending ($\sim 1640\text{ cm}^{-1}$) of water and overlapping contributions from aromatic rings and polysaccharides, thereby limiting diagnostic specificity of this region for anthocyanins [37].

An intense band at $\sim 1050\text{ cm}^{-1}$ is assigned to C–O and C–O–C stretching vibrations of carbohydrate moieties, including glycosidic linkages associated with flavonoid glycosides [31]. Additional bands at ~ 1516 , ~ 1450 , ~ 1270 and $\sim 1070\text{ cm}^{-1}$ are attributed to C–O–C and aromatic ring vibrations of flavonoid structures, although these features are often partially overlapped by signals from polysaccharides in plant matrices [31,38]. Finally, bands in the region $\sim 820\text{--}760\text{ cm}^{-1}$ have been tentatively associated with out-of-plane aromatic ring vibrations of anthocyanins [39]. Structural differences among anthocyanidins (e.g., delphinidin, malvidin, petunidin vs. cyanidin and peonidin) may induce subtle variations in band position and intensity, mainly related to the degree of hydroxylation and methoxylation of the aromatic rings [36,39].

The FTIR spectra of anthocyanins are therefore predominantly driven by the carbohydrate fraction rather than the structure of the chromophore, limiting the diagnostic specificity of the technique. This limitation is further increased by the complex system investigated in the present study, where overlapping contributions from the proteinaceous substrate and additional components (e.g., alum and/or other pigments) must be taken into account.

The application on parchment (collagen substrate) introduces dominant protein bands, namely Amide I ($\sim 1650\text{ cm}^{-1}$, C=O stretching) and Amide II ($\sim 1550\text{ cm}^{-1}$, N–H bending and C–N stretching), as well as weaker contributions in the $1230\text{--}1200\text{ cm}^{-1}$

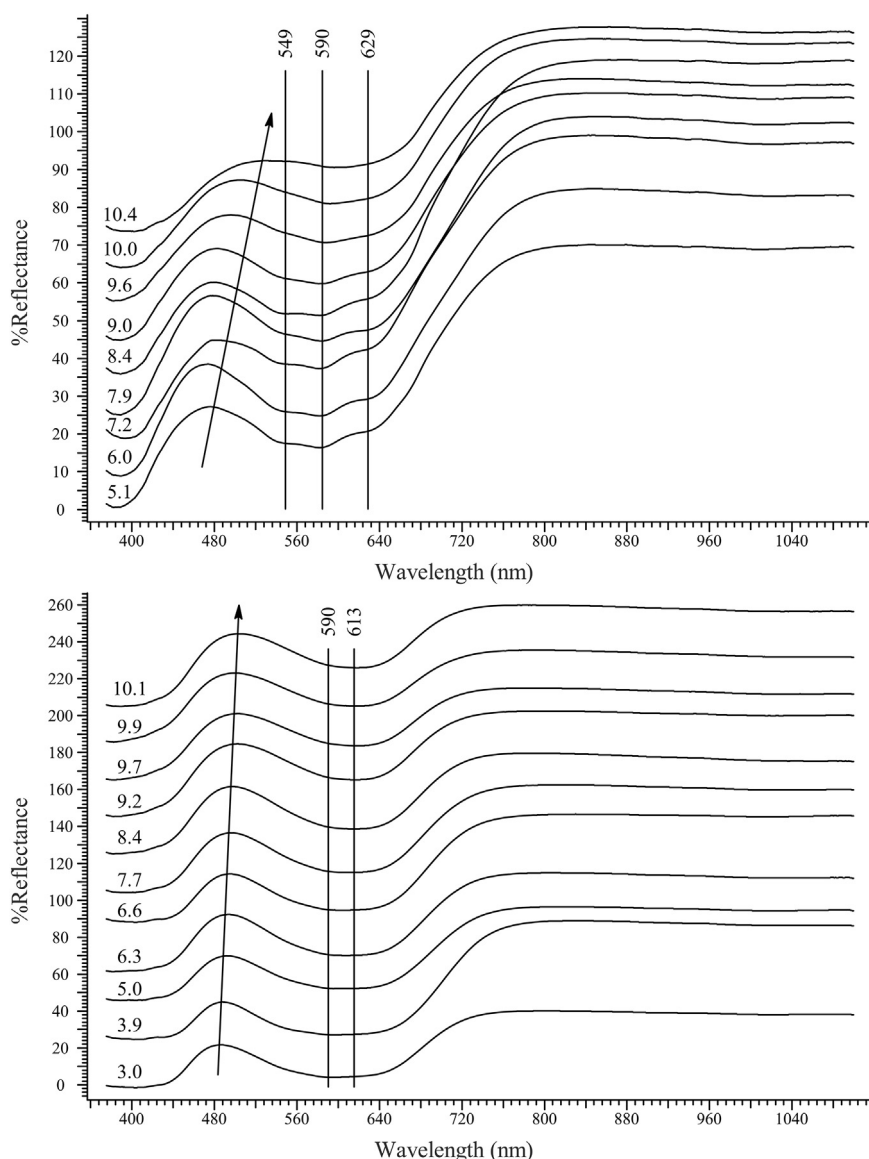


Fig. 2. FORS spectra of iris violet (top) and iris blue (bottom) at different pH values.

region (Amide III) [40]. The addition of alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) to obtain blue and green metal complexes introduces sulphate species characterised by bands typically observed at $\sim 1120\text{--}1100\text{ cm}^{-1}$ ($\nu_3 \text{SO}_4^{2-}$ asymmetric stretching), $\sim 1000\text{--}980\text{ cm}^{-1}$ (ν_1 symmetric stretching) and in the $650\text{--}600\text{ cm}^{-1}$ region (ν_4 bending) [41,42]. In the presence of verdigris, either in mixture or as an underlying layer, additional absorptions due to asymmetric and symmetric stretching of carboxylate groups (COO^-) are typically observed in the $\sim 1600\text{--}1550\text{ cm}^{-1}$ and $\sim 1450\text{--}1400\text{ cm}^{-1}$ ranges [43], further overlapping with both aromatic and protein-related bands.

Overall, FTIR interpretation of *Iris germanica*-derived dyes on parchment must be approached using a multicomponent spectral model, where overlapping contributions from carbohydrates, sulphates, metal-organic species, and collagen preclude unequivocal band attribution. Within this framework, the aim of FTIR analysis is not the identification of dye-specific spectral markers, but rather the recognition of characteristic spectral patterns resulting from the interaction of different components.

In this setting, the FTIR study was structured in three analytical steps in order to progressively assess the spectral contribution of each component:

- Pure dyes (iris violet, blue and green), prepared following historical recipes, were analysed in transmission mode using KBr pellets, in order to isolate and characterise the intrinsic spectral features of the dye systems, including dye-alum complexes in the case of the blue and green samples.
- Mock-up samples, consisting of the same dyes applied on parchment without binders, together with one sample of iris green mixed with gum Arabic and selected mixtures of iris green and verdigris with gum Arabic. The FTIR analysis was performed in reflectance mode. This step allowed evaluation of the influence of the substrate and of additional components.
- Historical samples, corresponding to iris green paint layers from illuminated manuscripts. The samples were analysed in reflectance mode under the same conditions, enabling comparison with the reference systems.

The main absorption bands typical of parchment, anthocyanin, sulphates and verdigris are summarised in Table 2, based on literature assignments. Although FTIR has limited capability for the direct identification of iris green, its use in the present study is justified by the complementary information it provides on the material system associated with *Iris germanica*-derived colourants on

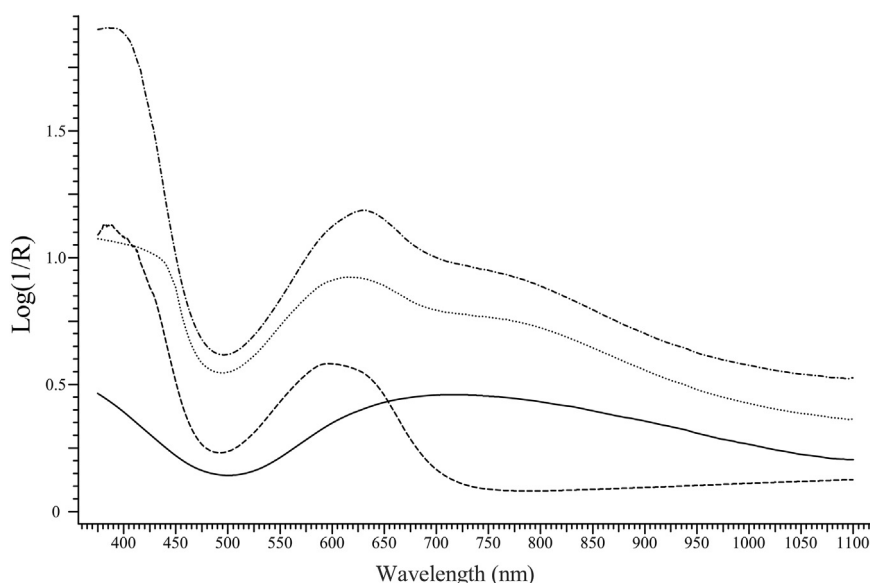


Fig. 3. FORS spectra in Log(1/R) coordinates of verdigris (solid line), iris green (dashed line), a mixture verdigris/iris green (dotted line) and iris green on top of verdigris (dashed-dotted line).

Table 2

Assignment of main infrared absorption bands (cm^{-1}) of parchment, anthocyanins, alum and verdigris.

Parchment		Anthocyanin (<i>Iris germanica</i>)		Sulphates (from alum)		Verdigris (Cu-acetate)	
Assignment [66,67]	cm^{-1}	Assignment [17,26,31,44,45]	cm^{-1}	Assignment [42,52,68,69]	cm^{-1}	Assignment [57,58]	cm^{-1}
$\nu(\text{CH}_3)$, $\nu(\text{CH}_2)$	2931, 2853	$\nu(\text{CH}_3/\text{CH}_2)$ asymmetric – aliphatic C–H stretch	2924–2854	-	-	$\nu(\text{CH}_3)$	2930
Amide I – C=O stretch	1652	$\nu(\text{C}=\text{C})$, $\nu(\text{C}=\text{O})$ aromatic / heterocyclic stretch	1655–1636	$\delta(\text{HOH})$ bending of hydrated sulphate / Al–OH	1640	-	-
Amide II – $\nu(\text{CN})+\delta(\text{NH})$	1538	$\nu(\text{C}=\text{C})$, $\nu(\text{CO})$ aromatic / carboxylate	1575–1500	-	-	$\nu_{\text{as}}(\text{COO})$	1574–1523
$\delta(\text{CH}_2)$ scissoring	1454	$\nu(\text{C}=\text{C})$ aromatic ring	1450	-	-	$\nu_{\text{s}}(\text{COO})$	1429
$\delta(\text{COH})$, $\delta(\text{NH}_2)$	1408	$\nu(\text{C}-\text{O}-\text{C})+\delta(\text{C}-\text{OH})$ carboxylate / phenolic O–H def.	1404	-	-	$\delta(\text{CH}_3)$	1354
Amide III – $\nu(\text{CN})+\delta(\text{NH})$	1279–1230	$\nu(\text{C}-\text{O})$, $\nu(\text{C}-\text{O}-\text{C})$ of glycosidic units	1270, 1210	-	-	-	-
$\nu(\text{CC})$ ring	1024	$\nu(\text{CC})$ aromatic ring / C–O stretch of phenols	1200, 1170	-	-	-	-
$\nu(\text{CC})$ skeletal	1081, 1062, 1034	$\nu(\text{C}-\text{O})$ of glycosides and sugars	1120, 1080, 1045	$\nu_3(\text{SO}_4)$ asym. stretch	1130–1135	$\rho(\text{CH}_3)$ methyl	1053–1028
-	-	-	-	$\nu_1(\text{SO}_4)$ sym. stretch (activated by Al-coordination)	~986	$\delta(\text{Cu}-\text{OH})$	977, 909
-	-	Anthocyanins ring vibrations	820–760	$\nu(\text{Al}-\text{O})$, $\delta(\text{Al}-\text{O})$	868, 685	$\nu(\text{CC})$	954, 935
-	-	-	-	$\nu_4(\text{SO}_4)+\nu(\text{Al}-\text{O})$ bending	613	-	-
-	-	-	-	-	-	$\delta(\text{OCO})$ carboxylate	679
-	-	-	-	-	-	$\pi(\text{COO})$	626–617
-	-	-	-	-	-	$\delta(\text{Cu}-\text{OH})$	548, 492

parchment. In particular, FTIR allows the investigation of interactions between the dye, recipe-related additives such as alum, and the collagen-based substrate, as well as the recognition of sulphate phases and possible transformation products formed during drying and ageing. This information is especially relevant for future studies on the stability of iris green and for preliminary assessments when more selective analytical techniques are unavailable or microsampling cannot be performed. The inclusion of the violet and blue forms was also relevant for FTIR interpretation, since it allowed the spectral contribution of the pure plant extract to be compared with that of the alum-containing systems. This comparison was essential for assessing the appearance and evolution of

sulphate-related bands, their interaction with the parchment substrate, and their possible transformation during drying and ageing. Therefore, the study of the three forms was not intended to expand the scope beyond iris green, but to reconstruct the material sequence leading to its preparation and to clarify the origin of the spectral features observed in the final dye system

4.2.1. Iris violet

The FTIR absorbance spectrum of the iris violet dye (Fig. 1, solid line) is dominated by carbohydrate-related features (e.g. free sugars and flavonoid glycosides). The broad O–H stretching band ($3600\text{--}3000\text{ cm}^{-1}$) reflects both water and hydroxyl groups from

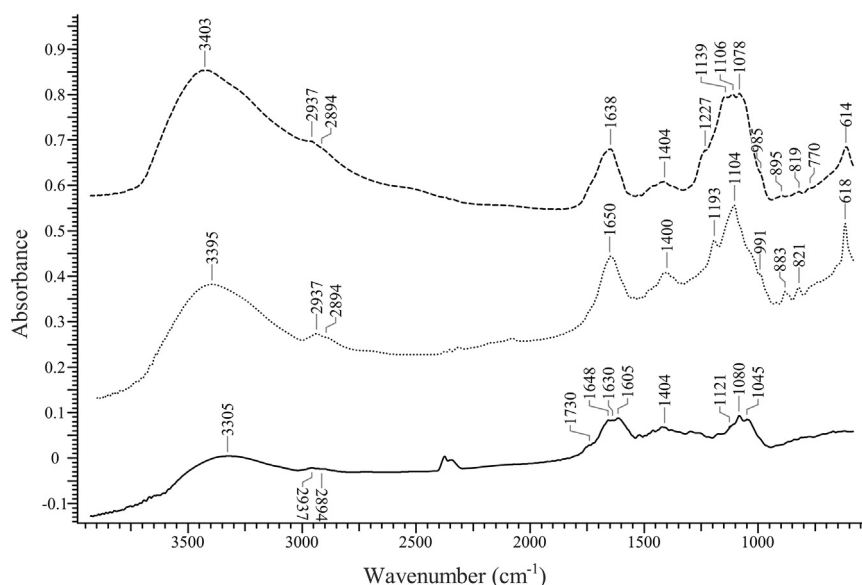


Fig. 4. FT-IR spectra of extracts of iris violet (solid line), iris blue (dashed line) and iris green (dotted line) measured in transmittance. Spectra are offset for clarity on Y axis.

sugars, while weak C–H stretching bands ($\sim 2930\text{--}2850\text{ cm}^{-1}$) are also observed. A broad and structured band in the $1650\text{--}1600\text{ cm}^{-1}$ region is predominantly attributed to H–O–H bending vibrations of water, with overlapping contributions from aromatic C=C skeletal modes of flavonoid compounds. The absence of a well-defined band in the $\sim 1700\text{ cm}^{-1}$ region suggests a limited contribution of non-conjugated C=O groups, consistent with the predominance of the quinoidal form of anthocyanins under these conditions [26].

A further band at $\sim 1404\text{ cm}^{-1}$ can be attributed to the C–OH deformation vibration, with an additional contribution from the symmetric O–C–O stretching of carboxylate groups [44,45]. However, partial overlap with bending modes of aliphatic or phenolic O–H groups cannot be excluded.

The most intense absorptions occur in the $1100\text{--}1000\text{ cm}^{-1}$ range (~ 1121 , ~ 1080 and $\sim 1045\text{ cm}^{-1}$), corresponding to C–O and C–O–C stretching vibrations of carbohydrate moieties [45,46], whose relative intensities can fluctuate according to sugar content [47]. This region is further characterised by multiple overlapping contributions from non-anthocyanic phenolic compounds and flavonoids (e.g., flavones and flavanols, commonly occurring in *Iris* species), which exhibit broad and poorly resolved aryl and alcoholic C–O stretching vibrations at $\sim 1210\text{--}1170$ to 1020 cm^{-1} [46].

The FT-IR spectrum of iris violet applied on parchment obtained in reflectance mode (Fig. 1) is dominated by the absorption of the collagen substrate – particularly the amide I, II and III bands ($1660\text{--}1550\text{--}1200\text{ cm}^{-1}$) and no evidence of the violet dye are visible.

4.2.2. Iris blue

The FTIR spectrum of iris blue dye, shown in Fig. 4 (dashed line), is dominated by sulphate-related modes, consistent with the addition of alum to promote anthocyanin-metal complexation. The interaction between anthocyanins and alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) leads to the formation of Al-anthocyanin complexes, which are responsible of the blue chromatic shift [17]. The resulting solid phase typically exhibits enhanced colour stability compared to the free anthocyanins, as the chelation of the metal centre restricts structural rearrangements and favours the quinoidal resonance form.

Upon dissolution, alum releases Al^{3+} and SO_4^{2-} ions into solution. When pH ranges between 5 and 7, partial hydrolysis of aluminium species may occur, leading to the formation of hy-

droxylated Al complexes [48]. During solvent evaporation, progressive concentration of the solution promotes supersaturation and favours the simultaneous occurrence of multiple processes resulting in multiple sulphate species incorporated into different phases – from more ordered crystalline salts (e.g., K_2SO_4) to aluminium-associated sulphate environments and poorly ordered or amorphous phases, depending on local conditions such as hydration, coordination and drying kinetics.

Sulphate IR signatures are typically observed through the asymmetric stretching mode (ν_3) in the $\sim 1150\text{--}1080\text{ cm}^{-1}$ region, the symmetric stretching mode (ν_1) around $\sim 990\text{--}980\text{ cm}^{-1}$ (activated under reduced symmetry), and the asymmetric bending mode (ν_4) in the $\sim 620\text{--}600\text{ cm}^{-1}$ range.

As expected, the composite feature of the $1200\text{--}970\text{ cm}^{-1}$ region suggests the presence of multiple sulphate-bearing species associated with different local environment. As shown in Fig. 4, a band at $\sim 1106\text{ cm}^{-1}$, together with the activation of the ν_1 mode at $\sim 990\text{ cm}^{-1}$ and a broad ν_4 band at $\sim 614\text{ cm}^{-1}$, indicates sulphate tetrahedra in distorted, low-symmetry environments [49–51]. This distortion is likely related to coordination to aluminium, hydrogen bonding and/or local structural heterogeneity, rather than the presence of fully crystalline K_2SO_4 [52] and is typically associated with a broadening of the asymmetric bending mode at $\sim 620\text{--}600\text{ cm}^{-1}$, as observed in the spectrum of iris blue.

An intense band at $\sim 1139\text{ cm}^{-1}$ can be interpreted as the upper component of a split ν_3 envelope, reflecting further distortion and the presence of non-equivalent sulphate sites with different degrees of hydration, coordination and structural order [41]. In Al-anthocyanin complexes, coordination is expected to affect bands associated with oxygen-containing functional groups, particularly phenolic C–O vibrations in the $\sim 1270\text{--}1200\text{ cm}^{-1}$ region and glycosidic/carbohydrate C–O–C vibrations in the $\sim 1100\text{--}1000\text{ cm}^{-1}$ region [26,48]. These effects mainly result in band broadening and minor shifts, which may contribute to the overall spectral profile in this region, although to a lesser extent compared to the dominant sulphate-related absorptions.

Additional bands at ~ 895 , ~ 819 and $\sim 770\text{ cm}^{-1}$ can be attributed to out-of-plane vibrations, possibly associated with sulphate-related modes and/or aromatic ring vibrations of the organic fraction compatible with flavonoids/anthocyanin residues [31].

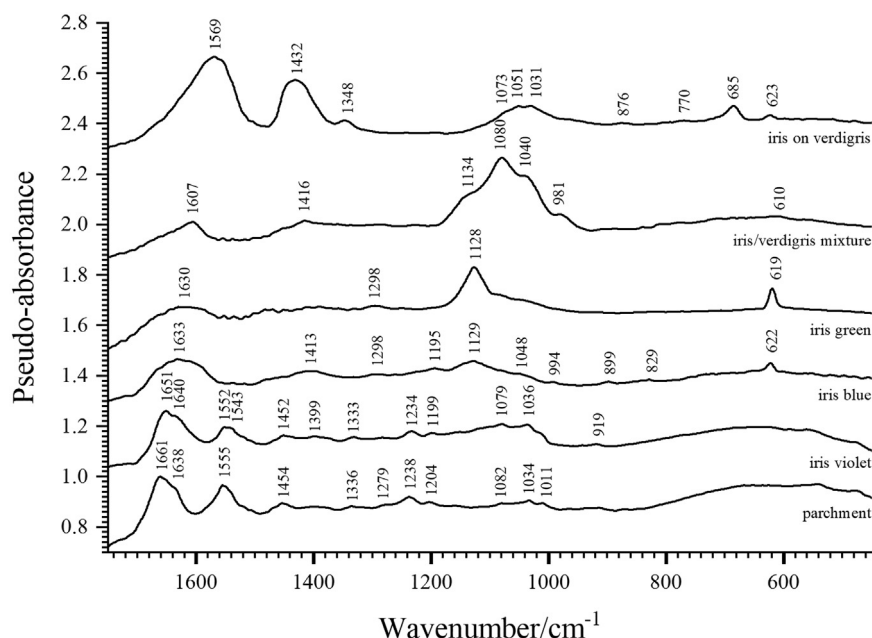


Fig. 5. FT-IR spectra of mock-ups on parchment of iris violet, iris blue, iris green, iris/verdigris mixture, iris on verdigris and of parchment, measured in reflectance and converted in pseudo-absorbance. Spectra are offset for clarity on Y axis.

When iris blue is applied on parchment (Fig. 5), the FTIR spectrum shows significant changes, particularly in the 1200–1000 cm^{-1} region, where a broad band centred at $\sim 1126 \text{ cm}^{-1}$ is observed. This feature, together with a well-defined band at $\sim 623 \text{ cm}^{-1}$, is consistent with the presence of more ordered sulphate phases, likely including crystalline K_2SO_4 [53]. In this context, the porous collagen substrate may accelerate water loss and act as a heterogeneous nucleation site, promoting the late-stage precipitation of free $\text{K}^+/\text{SO}_4^{2-}$ as well-ordered K_2SO_4 [54].

At the same time, the occurrence of the ν_1 symmetric stretching mode band around $\sim 990 \text{ cm}^{-1}$, indicates the coexistence of sulphate species in less symmetric environments, likely associated with coordination to aluminium or structural disorder within amorphous phases. Additional bands at ~ 896 , ~ 819 and $\sim 770 \text{ cm}^{-1}$ can be attributed to out-of-plane vibrations, possibly associated with sulphate-related modes and/or aromatic ring vibrations of the organic fraction. Overall, these spectral changes suggest a transition toward a more structured sulphate environment upon drying on the parchment substrate, likely promoted by solvent evaporation and heterogeneous nucleation.

4.2.3. Iris green

The FT-IR spectrum of iris green extract is shown in Fig. 4 (dotted line). Sulphate-associated absorption bands are visible at 618 cm^{-1} and within the 1200–970 cm^{-1} region, where the contributions from carbohydrate C–O/C–O–C modes should be also considered. The broadening of the 618 cm^{-1} toward lower frequencies, together with the presence of a well-defined band at 991 cm^{-1} (consistent with partial activation of the ν_1 symmetric stretch) suggests the coexistence of crystalline K_2SO_4 and basic aluminium sulphate phases [42]. The bending modes of the water molecule for the hydrated Al-sulphates occur around 1650 cm^{-1} .

When iris green is applied on parchment (Fig. 5), the crystallisation of K_2SO_4 is favoured by the substrate and sharper absorption bands at 1126 cm^{-1} and 619 cm^{-1} are visible.

The FTIR spectrum of iris–gum Arabic mixture was also investigated in order to assess the potential influence of the organic binder on the spectral signature of iris green. The spectrum is largely dominated by the polysaccharide component, with the

most intense features observed in the 1200–1000 cm^{-1} region, corresponding to overlapping C–O and C–O–C stretching vibrations of glycosidic linkages. Additional contributions in the 1425–1400 cm^{-1} range can be attributed to CH_2 bending and C–OH deformation modes of the polysaccharide backbone [55,56]. No distinct bands that can be unambiguously assigned to the iris-based colourant are resolved, while sulphate-related features are absent or not clearly distinguishable. Notably, sulphate-related bands, typically expected around ~ 620 – 610 cm^{-1} , are not detectable. This absence is interpreted as the result of combined effects, including low concentration, structural disorder, and spectral masking induced by the dominant organic matrix, rather than the actual absence of sulphate species. This behaviour suggests that, in such systems, the FTIR response is primarily governed by the organic binder, which effectively masks the weaker and less specific vibrational signatures of the dye.

4.2.4. Mixtures of verdigris and iris green

The FT-IR spectra of verdigris and iris green mixtures applied on parchment (Fig. 5) are predominantly governed by the spectral response of gum Arabic, whose characteristic polysaccharide features dominate the overall profile. Within this framework, a relative enhancement of the 1600–1400 cm^{-1} region can be observed, which is consistent with the presence of verdigris and can be attributed to the asymmetric and symmetric stretching of carboxylate groups [57,58]. Conversely, no distinct spectral features attributable to the iris-based dye are detectable, indicating that its contribution is effectively masked within this complex system.

On the other hand, the spectrum of the iris layer applied over verdigris is characterised by the dominant contribution of the copper-based pigment. The bands at ~ 1569 and $\sim 1432 \text{ cm}^{-1}$ are assigned to the asymmetric and symmetric stretching vibrations of carboxylate groups (COO^-), which are diagnostic of verdigris, while the feature at $\sim 1348 \text{ cm}^{-1}$ can be attributed to additional acetate-related vibrations. In the 1070–1030 cm^{-1} region, a structured envelope is observed, likely resulting from the overlap between C–O vibrations of the acetate groups and the ν_3 asymmetric stretching of sulphate species. The presence of sulphates is further supported by the band at $\sim 623 \text{ cm}^{-1}$, assigned to the ν_4 bending mode of

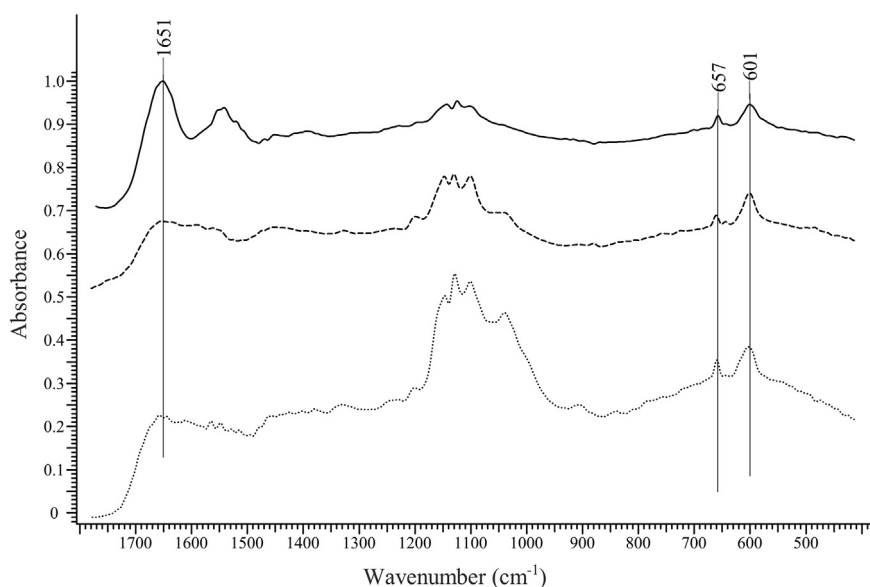


Fig. 6. FT-IR spectra of green areas in the *Collectio antiquitatum* (solid line), the *Matricola dell'arte della seta* (dashed line) and the *Ercole Senofontio* (dotted line), measured in reflectance and converted in pseudo-absorbance. Spectra are offset for clarity on Y axis.

SO_4^{2-} , while the signal at $\sim 685 \text{ cm}^{-1}$ is consistent with Cu–O vibrations of the verdigris structure. Minor bands at ~ 876 and $\sim 770 \text{ cm}^{-1}$ may be related to secondary components, such as carbonates or out-of-plane vibrations of aromatic systems.

4.2.5. Application of FT-IR spectroscopy to historical manuscripts

The investigation of iris green found in three Renaissance codices – the *Collectio antiquitatum* (ms. alfa.I.5.15, Biblioteca Estense Universitaria, Modena, Italy), the *Matricola dell'arte della seta* (Archivio di Stato, Naples, Italy) and the *Ercole Senofontio* (ms. B.P. 1099, Biblioteca Civica, Padova, Italy) revealed distinctive spectral features compared with the mock-up samples. The FTIR spectrum of green layers applied on parchment (Fig. 6) is characterised by a composite signature dominated by the proteinaceous substrate and sulphate-related phases. The region $1700\text{--}1500 \text{ cm}^{-1}$ shows the typical amide I ($\sim 1650\text{--}1635 \text{ cm}^{-1}$) and amide II ($\sim 1555\text{--}1540 \text{ cm}^{-1}$) bands of collagen, partially overlapped by water bending contributions. The $1200\text{--}1000 \text{ cm}^{-1}$ region is characterised by an intense and structured absorption band centred at $\sim 1123 \text{ cm}^{-1}$, together with further bands at ~ 1141 , ~ 1095 and $\sim 1034 \text{ cm}^{-1}$, that can be attributed to the ν_3 asymmetric stretching of SO_4^{2-} in multiple local environments. The presence of a well-defined band at $\sim 603 \text{ cm}^{-1}$, together with contributions at ~ 642 and $\sim 658 \text{ cm}^{-1}$, supports the occurrence of sulphate phases with different degrees of structural order. Additional bands at $\sim 876 \text{ cm}^{-1}$ and in the $1450\text{--}1400 \text{ cm}^{-1}$ region suggest the presence of calcium carbonate, likely related to parchment processing residues [59]. No evidence of verdigris can be observed.

The possible presence of a polysaccharide binder, such as gum Arabic, was also considered. However, the characteristic carboxylate bands of gum Arabic ($\sim 1610\text{--}1600$ and $\sim 1420\text{--}1410 \text{ cm}^{-1}$) are not distinctly resolved, as they are largely overlapped by the intense amide bands of the collagen substrate and by possible carbonate contributions. Similarly, the $1200\text{--}1000 \text{ cm}^{-1}$ region, where gum Arabic typically exhibits strong C–O and C–O–C stretching vibrations, is dominated by sulphate absorptions. Based on the results obtained from the reference mixtures, the presence of gum Arabic would be expected to exert a dominant influence on the FTIR spectral profile, leading to clearly recognisable polysaccharide features and to the masking of contributions from other components. Since such a spectral pattern is not observed in the anal-

ysed samples, it can be reasonably inferred that a polysaccharide binder is either absent or present in negligible amounts, and therefore does not play a significant role in the spectral response.

The most interesting aspect of the original paint layers concerns the complex sulphate signature.

In particular, the presence of a high-wavenumber component at $\sim 1140 \text{ cm}^{-1}$, together with multiple of bands in the $650\text{--}600 \text{ cm}^{-1}$ region, suggests the coexistence of sulphate species in non-equivalent structural environments. These features are particularly consistent with the presence of mixed K–Ca sulphate hydrates phases (e.g. syngenite-like phase, $\text{K}_2\text{Ca}(\text{SO}_4)_2 \cdot \text{H}_2\text{O}$) that can form through the interaction of migrating SO_4^{2-} and K^+ ions (released from alum decomposition) with Ca^{2+} ions from the substrate, originating from residues of lime-based treatments carried out during parchment manufacture [59]. Although the formation of mixed K–Ca sulphate phases during the initial drying stage cannot be entirely excluded, their absence in the modern reference sample suggests that the syngenite-like signature observed in the historical parchment is more likely related to long-term post-depositional processes, possibly driven by repeated humidity cycles and progressive salt reorganisation over time [52,60,61].

In conclusion, although FTIR cannot be regarded as a stand-alone technique for the direct identification of iris green, it can provide relevant information on materials and reaction products associated with the dye. In particular, the identification of alum-derived sulphate phases, together with the investigation of chemical processes occurring during drying and long-term ageing on parchment, provides complementary information that is essential for a broader understanding of *Iris germanica*-derived colourants and their application on parchment.

Despite FTIR signatures cannot be considered diagnostic for dye identification on their own, sulphate speciation and its interaction with the parchment substrate may retain valuable information on the long-term chemical evolution of iris-based colour layers. Within this framework, the comparison between historical samples and modern mock-ups highlights the potential of FTIR as a tool for assessing material compatibility and post-depositional transformations, rather than for direct molecular identification. Future studies should therefore focus on ageing experiments on parchment-based mock-ups, including humidity cycling, light exposure, and controlled variations in calcium-rich surface residues, in order to

evaluate whether the complex sulphate signatures observed in historical samples can be reproduced and related to long-term alteration pathways. This would provide useful indications for assessing the stability of iris green and for defining more appropriate conservation strategies.

4.3. Spectrofluorimetry analysis (FOMF)

Contrarily to UV-visible absorption, reference data for the fluorescence of petals or extracts of *Iris germanica* are not available. As previously stated, analytical evidence from earlier studies [21,22] suggests that iris dyes are composed primarily of anthocyanins along with copigmentation agents. However, anthocyanins are reported to be poorly fluorescent [62]. Therefore, to obtain meaningful information using this technique, the measurement conditions must be optimised to maximise the inherently weak emission. We used an edge filter with cutoff at 442 nm in order to remove the Rayleigh emission provoked by the elastic scattering of the 385 nm LED source. In this way it was possible to collect the fluorescence spectra of the three dyes with long excitation times, without the risk of overloading the detector.

The comparison with the scientific literature is challenging, as most available data were obtained in solution, which differs from the method used in our study. Among the few comparable investigations, Iriel and Lagorio [63] examined the optical properties of *Rhododendron indicum* petals, whose colouration is due to anthocyanins. The authors reported one emission band between 400 and 500 nm, attributed to hydroxycinnamic-type phenolic compounds, and another band at 624 nm, attributed to anthocyanins.

When applying molecular fluorescence to a solid sample, it is important to compare the spectrum of the dyes with that of the parchment, since most of the fluorescence emission originates from the parchment itself. This is particularly relevant given that the mock-up paints lack a body, being composed solely of dyes rather than pigments or lakes.

Although the overall fluorescence emission of the iris dyes was low in all three cases, specific spectral features could still be identified, providing a means to detect the dyes non-invasively.

4.3.1. Iris violet

The fluorescence spectrum of iris violet has an emission band centred at ca. 514 nm with a shoulder at ca. 543 nm (Fig. 1, solid line). According to Iriel and Lagorio [63], this band could be due to hydroxycinnamic-type phenolic compounds.

4.3.2. Iris blue

The spectrum of iris blue has a small band at ca. 680 nm (Fig. 7, dashed line), which can be attributed to anthocyanins.

4.3.3. Iris green

The spectrum of iris green (Fig. 7, dotted line) is the richest in spectral features. It shows a band with high fluorescence at 474 nm, possibly due to other phenolic compounds, and a significant band centred at 720 nm, which could be attributed to anthocyanins. This band could be a useful feature to specifically identify iris green in paints. (Fig. 6, 8)

4.3.4. Mixtures of verdigris and iris green

Unlike the FORS spectrum of the mixture, the FOMF response does not permit the identification the mixture. This is because the emission band of iris green, at 720 nm, coincides with the absorption band of verdigris, which is centred at the same position, resulting in complete absorption of the emission. Consequently, the presence of iris green cannot be detected with FOMF when it is mixed with verdigris.

4.4. Surface enhanced Raman spectroscopy analysis (SERS)

The SERS spectra shown below (Fig. 8) are, to the best of our knowledge, the first published spectra of extracts from the petals of *Iris germanica*. The three spectra exhibit similar features, and the corresponding peak assignments are reported in Table 3. Spectra were interpreted based on the assignments provided by Merlin et al. [64] and by Zaffino et al. [65] in their studies on anthocyanins and anthocyanidins. It should be noted, however, that molecules other than anthocyanins may also contribute to the observed spectra.

4.4.1. Iris violet

The SERS spectrum of iris violet is characterised by strong and medium peaks at 1262, 1291, 1431, 1551 and 1608 cm^{-1} , with minor peaks at 431, 480, 528, 550, 627, 738, 807, 878, 1214, 1331 and 1634 cm^{-1} .

4.4.2. Iris blue

Despite the coordination of anthocyanins with Al^{3+} , the SERS spectrum of iris blue is very similar to the previous. The main difference is the shift of the band at 1551 cm^{-1} to 1537 cm^{-1} .

4.4.3. Iris green

The SERS spectrum of iris green is dominated by two peaks occurring at 1527 and 1597 cm^{-1} , the other features being nearly unaltered.

4.4.4. Mixtures of verdigris and iris green

In this case, given the high spatial resolution of the Raman technique, it is easy to recognise the presence – and therefore to obtain a selective identification – of verdigris mixed with iris green.

4.5. Elemental analysis

Elemental analysis, typically performed by X-ray fluorescence spectrometry (XRF), does not directly detect the elements in iris dyes. However, the contextual identification of Al, K, and S can indicate the presence of alum, and thus suggest the use of iris blue and iris green. Indeed, XRF analysis has not been performed on the mock-ups but it was performed on the manuscripts cited in this work, in order to verify whether it was the dye or a mixed iris green/copper pigment. In some cases, in fact, iris green may be mixed with or superimposed on verdigris, as discussed previously. In such instances, the detection of Cu would be expected, suggesting either the exclusive presence of verdigris or other copper-based green pigments. In the XRF spectrum reported in Figure S1, taken on the *Commissione dogale del Doge Andrea Gritti* (16th century, Torino, Archivio di Stato) from a light green paint containing iris green (red line), it is possible to see that the peak of Cu is lower – but not absent – than in a green paint containing verdigris (black line). Moreover, at low energy values the peaks of Al and K highlight the presence of alum, while these elements are absent in the paint with verdigris. The presence of S cannot be ascertained due to the positive interference of the M_{α} peak of Pb on the K_{α} peak of S. The possible mixture or superimposition of iris green and verdigris, however, should be critically interpreted in conjunction with FORS and FOMF evidence.

4.6. Identification on manuscripts

As previously stated in the Research aims section, during different analytical sessions on illuminated manuscripts dated between 1450 and 1585, the list of which is reported in Table 1, in several FORS measurements on green areas we obtained spectral responses

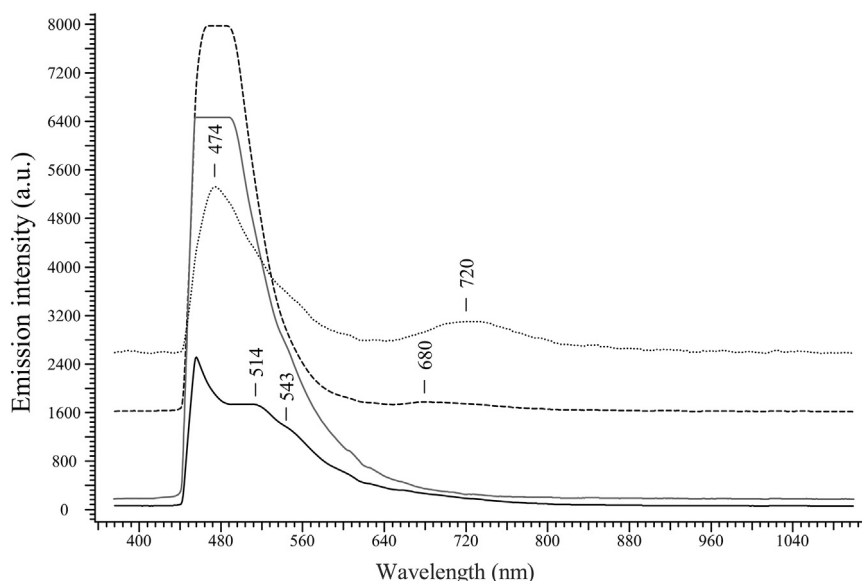


Fig. 7. FOMF spectra of iris violet (solid black line), iris blue (dashed black line), iris green (dotted black line) and parchment (solid grey line). Spectra are offset for clarity on Y axis.

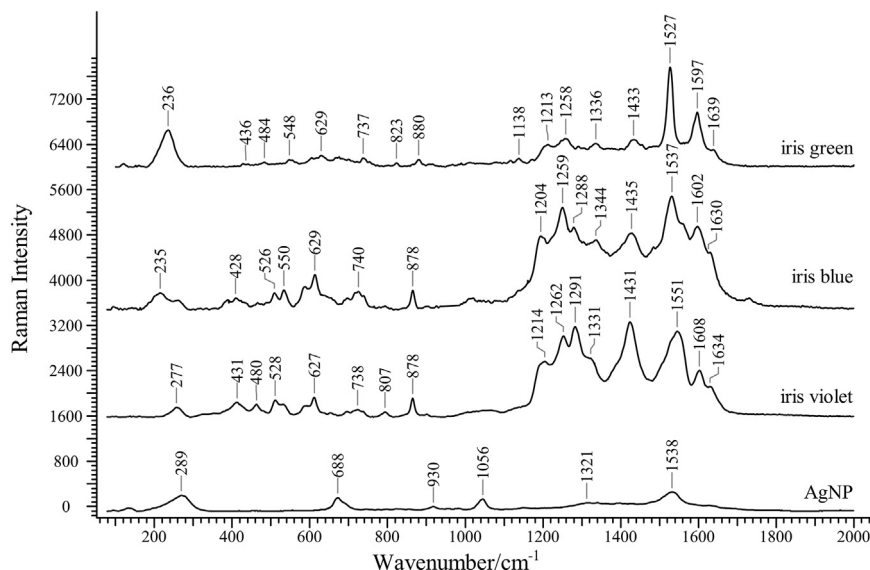


Fig. 8. SER spectra of iris violet (solid line), iris blue (dashed line) and iris green (dotted line). Spectra are offset for clarity on Y axis.

which we were unable to assign to known green colourants. Using the spectral features of the FORS analysis highlighted in the present study, the identification of iris green is now suggested in this series of manuscripts produced between the late Middle Ages and the Renaissance, in agreement with the period during which most of the ancient recipes were published. In particular, the dye was identified in three of the volumes produced and decorated by Felice Feliciano (*Ercole Senofontio, Collectio antiquitatum* and *Epigrammata*), who used iris green to prepare a green ink but also a green paint with low hiding power. The green painted areas contained in these volumes were very useful for this study, as they could be considered sort of “standard” paints. (Table 2, 3)

Based on this limited, but significant, number of identifications, the use of iris green appears to span the Late Middle Ages and the Renaissance. However, the earliest documented recipes date back to the 12th century (Theophilus Presbiter) [2], suggesting that more comprehensive analyses across a wider corpus of manuscripts are warranted.

Although the characteristic FTIR bands of anthocyanins cannot be unambiguously identified due to extensive spectral overlap with the substrate and other components, the combined analysis of violet, blue and green dyes (in transmission mode), their corresponding mock-ups on parchment, and historical manuscript samples (both in reflectance mode) reveals the presence of consistent spectral patterns. These patterns are primarily defined by the relative contributions and evolution of sulphate-related modes, their degree of structural order, and their interaction with the parchment substrate. In particular, the progressive modification of the ν_3 sulphate envelope in the 1200–1000 cm^{-1} region, together with the behaviour of the ν_4 band ($\sim 620 \text{ cm}^{-1}$) and the activation of the ν_1 mode ($\sim 990 \text{ cm}^{-1}$), provides a diagnostic framework for characterising iris-based colourants. Therefore, even in the absence of clearly resolved anthocyanin bands, FTIR analysis allows the identification of specific spectral signatures associated with iris-derived colour layers, based on the overall spectral pattern rather than on individual marker bands.

Table 3
List of Raman peaks.

Raman shift (cm ⁻¹)	Assignment*	Iris violet**	Iris blue**	Iris green**
431	Γ(CC)	w	w	vw
480	Γ(CC)	w	vw	vw
528	δ(CC)	m	w	vw
550	δ(CC)	w	m	w
627	δ(CC)	m	m	vw
738	γ(CH)	w	m	vw
807	γ(CH)	w	vw	vw
878	γ(CH)	m	m	w
1214	δ(OH)	m	1204m	w
1262	ν(CO)	s	s	m
1291	ν(CO)/ν(CC)/δ(CCC)	s	m	vw
1331	ν(CC)	sh	m	m
1431	ν(CC)	vs	s	m
1551	ν(CC)	vs	1537s	1527vs
1608	ν(CC)	m	1602m	1597s
1634	ν(CO)/ν(CC)	sh	sh	sh

* ν, stretching; δ, in-plane bending; γ, out-of-plane bending; Γ, skeleton out-of-plane bending.

** vw, very weak; w, weak; m, medium; s, strong; vs, very strong; sh, shoulder.

XRF analysis was systematically carried out on the manuscripts analysed in this work (listed in Table S1). The results evidenced in all cases a low but significant amount of Cu, suggesting that iris green was used on top of a copper pigment, rather than mixed to it, a feature compatible with most of the recipes described in the introduction. Moreover, the content of Al and K in the green paints was higher than in the parchment, confirming in all cases the presence of alum, while it was difficult to estimate the content of S, due to its content in parchment and sometimes to the occurrence of Pb, which M_α line overlaps with the K lines of S.

5. Conclusions

Ultimately, the four techniques employed in this study enable the selective identification of dyes extractable from *Iris germanica*, in particular the iris green referenced in ancient technical treatises. FORS and FT-IR in reflectance mode showed to be reliable for obtaining a totally non-invasive identification; SERS added the possibility to obtain a fingerprint identification, though micro-invasive and destructive. Furthermore, the combined application of FORS and FOMF analyses may allow detection of the dye even when it is mixed with verdigris, as suggested by the treatises themselves. Additionally, relevant information can be obtained by means of XRF analysis, in order to verify whether iris green was used (1) as a pure juice, (2) in mixture with verdigris or (3) on top of a verdigris layer.

The historical interest of iris green also lies in its possible use as an organic alternative to more reactive green pigments, particularly in manuscript illumination, where delicate supports such as parchment could be affected by potentially aggressive or unstable materials. In this perspective, the study of iris green was not limited to the identification of the chromophore alone, but also considered the behaviour of the whole paint system. Although FTIR does not provide a direct identification of the anthocyanin dye, it proved useful for investigating the interactions between the colourant, recipe-related additives such as alum, and the parchment substrate. The recognition of alum-derived sulphate phases and other associated spectral features provides complementary information on drying processes, substrate-colourant interactions and possible long-term alteration pathways. These aspects are particularly relevant for understanding the stability of iris green on parchment and may contribute to defining more appropriate conservation strategies for illuminated manuscripts.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.culher.2026.10.012](https://doi.org/10.1016/j.culher.2026.10.012).

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