**Perspective**

# Compound Specific Radiocarbon (14C) Dating of Our Colorful Past: from Theory to Practice

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For generations, humanity has preserved customs, places, objects, artistic expressions, and values – our cultural heritage. The use of color on cultural heritage objects is ubiquitous and found on artefacts from prehistoric rock art to present day contemporary artworks. The chemical identification of the colored materials used within an artwork often provides information about the work's origin. At the simplest level, a comparison of the materials present with information on their first date of discovery indicates the earliest possible period in which the colored artefact was created. More precise constraints on the date of creation can be provided by radiocarbon (14C) dating, however until today no such analysis has ever been conducted on the compounds responsible for the object's color. The analysis of natural organic dyes and pigments is challenging, as the limited sampling access, their low concentrations and presence in highly complex matrix, are all major challenges to be overcome. The separation of intermingled carbon sources is without question the most difficult problem, yet feasible with the help of compound specific radiocarbon analysis (CSRA). Here, we discuss the potential of radiocarbon dating isolated natural organic dyes and pigments and explore new routes to date cultural heritage objects.

**Keywords:** analytical methods, liquid chromatography, heritage sciences, natural organic dyes, radiocarbon (14C) dating.

## 1. Radiocarbon (14C) Dating and Heritage Science

Heritage science is a cross-disciplinary field of scientific research that is often described as the application of science to cultural heritage materials. From the traditional triangle linking solely conservation, science and art history, heritage science is growing into a much wider intersected field connecting objects, people, places, and time. The International Centre for the Study of the Preservation and Restoration of Cultural Property, commonly known as ICCROM, defines heritage science as an umbrella term: *'Drawing on diverse humanities, sciences and engineering disciplines, heritage science is an umbrella term encompassing all forms of scientific inquiry into human works, and the combined works of nature and humans, that are of value to people. Heritage science focuses on enhancing the understanding, care, sustainable use and management of tangible and intangible heritage so it can enrich people's lives: both today and in the future.'* Many journals are dedicated to heritage science, but this field is also of interest for journals with broader scope as highlighted by the recent issue featuring heritage science in *Angewandte Chemie*.[1]

Radiocarbon dating has a large role to play in providing missing information about an artwork's historical context. Such information, however, can only be obtained by sacrificing a sample from the object, a critical step owing to the unique and irreplaceable nature of art objects. The first consideration in this approach is sample size. Since the initial radiometric methods of the 1940s, which measured the radiation produced by decaying 14C-atoms and necessitated grams of carbon,[2] to the advent of accelerator mass spectrometry (AMS) instruments in the 1970s,[3,4]

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which allowed the direct counting of $^{14}$C-atoms in a sample bearing as little as 1 mg carbon, sample size were successfully reduced by three orders of magnitude. With the development of gas ion sources in the late 1980s,[5,6] sample material could be measured as carbon dioxide. A new milestone was reached early 2000s namely 10 µg C proved to be sufficient in producing a radiocarbon age.[7–9]

The ability to date minute samples led scientists to reconsider research fields that were initially beyond the reach of $^{14}$C dating, for example heritage science.[10] From early cave paintings[11–14] to contemporary artworks,[15–17] radiocarbon dating has been widely solicited to provide missing information about an artistic object's age. It has actually been dubbed one of the greatest advances in the authentication of works of art.[18] Its extreme sensitivity to post-1950 material provides indisputable evidence in forgery cases.[19–24] Following above-ground thermonuclear weapon testing conducted in the 1960's, the natural atmospheric $^{14}$C concentration doubled, an effect known as 'bomb peak'. The result is a distinctive $^{14}$C signal to be found in any growing organism and so may be used as a marker for late 20th century organic materials.

For most artworks, objects, and materials from our collective cultural heritage, $^{14}$C dating has generally focused on the support material, as it usually offers sufficient sampling opportunities. Recently, the down-scaling of the sample size requirement has supported the development of new strategies and led to the exploration of new materials for $^{14}$C dating of artworks. Although predicted by *Stulik* and *Donahue* in the 1990s,[25] $^{14}$C dating of the natural organic binder (oil, natural resin, plant gums, animal glues, *etc.*) in paint layers has only lately been exemplified in canvas paintings.[20,26] The proof of concept was demonstrated on a bulk sample of inorganic pigmented paint sampled from *Rederer*'s portrait of *Margrit* (see *Figure 1*). The application potential was thereafter highlighted in a forgery case, where the collected paint sample weighed no more than 160 µg and sufficed to reveal a forger's strategy in recycling an older canvas support.[20] Additionally, the possibility to date specific pigments, such as one of the most common artistic pigments lead white, a basic lead carbonate composed mainly of the lead carbonates hydrocerussite ($2PbCO_3 \cdot Pb(OH)_2$) and cerussite ($PbCO_3$), was demonstrated.[28–37] This development represents another breakthrough with massive implications as lead white has been the most extensively used white pigment by artists worldwide since Antiquity up to the 20th century. With the continuous decrease in sample size, sampling requirements for $^{14}$C dating now align with those routinely taken for material analysis and cross section sampling, substantially increasing the number of cultural objects that can be ethically sampled. However, despite successful demonstrations

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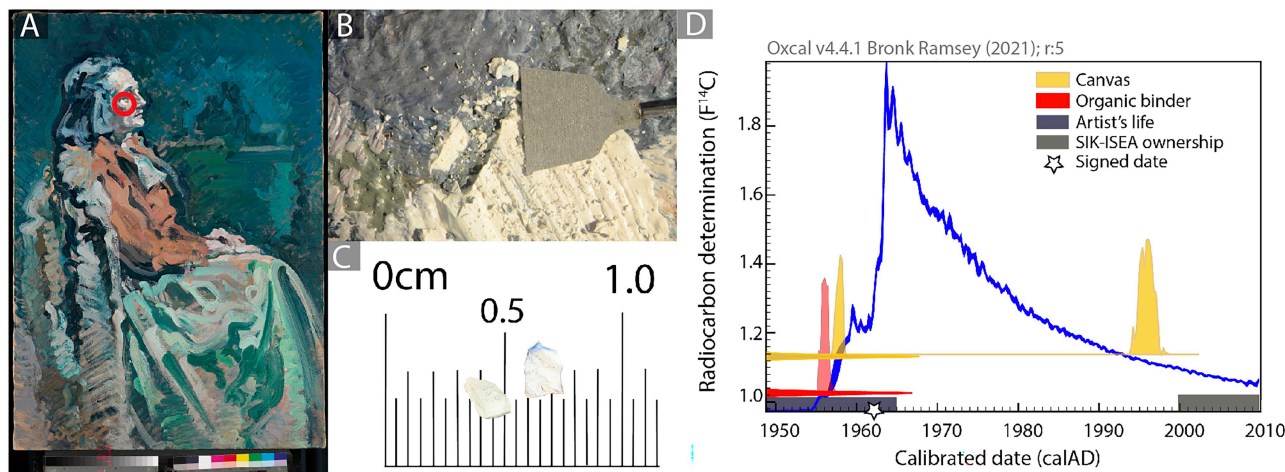
**Figure 1.** A) Bildnis Margrit mit roter Jacke und Konzertkleid, *Franz Rederer*, 1962, oil on canvas, 100×140 cm, Photograph: SIK-ISEA (*Philipp Hitz*). Sampling locations are highlighted in red. B) The close-up depicts the sampling process and the respective damage after sampling, while C) represents the actual sampled paint amount. D) Using the calibration software OxCal v.4.4.1, the calibration curve (blue) allows the conversion of the measured radiocarbon ages on the canvas and paint sample with their uncertainty on the ordinate axis to the respective calendar ages on the abscissa axis, which is indicated by the histograms (95.4% probability). Both the canvas and paint predate the signed date by a few years. Material originating from *Hendriks*' doctoral thesis.[27]

of these techniques, no radiocarbon laboratory offers the routine analysis of either the paint binder or lead white pigment, which raises the question as to why not.

The theoretical assumption that underlies the interpretation of ^{14}C ages gained from testing the natural organic binder or lead white pigment is that the sample represents a single carbon source. In reality, the inescapable presence of additional carbon sources within the organic binder such as carbonate pigments or fillers, organic pigments, and carbon black all violate this initial assumption. Carbonates typically carry a geological ^{14}C signature (*i.e.*, carbon essentially free of ^{14}C), whereas natural organic dyes bear the atmospheric ^{14}C signal of their time of growth, or in contrast, if of synthetic origin are ^{14}C depleted since they are formed from coal-tar products.[38] Carbon black, a common black pigment produced from charring of organic matter, has allowed the dating of rock art in prehistoric caves around the world,[14,39] but its interpretation in paint formulation is less unequivocal. The artist could have used contemporary material, although perhaps with an inbuilt age of the charred organic material (wood or bone), but they may have also utilized a carbon black created using a depleted natural gas in the combustion step. Carbonates may be readily removed by acid dissolution, however the separation of carbon black and/or organic pigments, whether synthetic or natural, from the sample material on a macroscopic level is difficult, if not impossible to

achieve fully. Their presence must be excluded by spectroscopic or microscopic analyses as even trace amounts may strongly influence the outcome of the results. Sample selection and detailed chemical characterization is therefore a crucial parameter in this approach, as it requires considerable expertise, a detailed knowledge of the materials present, and an understanding of all the possible pigment interferences.

The overall history of the object must furthermore be considered from its creation until its current state today. Most artworks are likely to present intricately complex surfaces, carrying multiple paint layers, as well as phases added during conservation and restoration interventions, both carried out historically or in modern times. Many materials, from waxes to proteins, natural and synthetic polymers, and resins, may have been added to the original paint materials. All of these may carry different isotopic signatures, thereby adding to the challenge of finding a suitable sample. Older apparent ages have on occasion been reported and linked to the occurrence of unknown conservation material contamination bearing a depleted ^{14}C signature.[21,40] Although no official accurate survey exists, it is estimated that a significant proportion of paintings in museum collections have undergone conservation and restoration treatments. This implies that upon inadequate sample preparation procedures, *i.e.*, incomplete removal of restoration products, the measured ^{14}C date may be significantly biased.[41] In

addition to older ages caused by $^{14}$C depleted petroleum-based conservation materials, younger ages can also result, for instance when a naturally-derived substance such as beeswax has been used.

The following selected examples reveal the difficulty in localizing materials meeting the sampling criteria suitability, *i.e.*, absence of other carbon material other than the natural organic binder. During the investigation of an oil painted wooden panel, the presence of *Laropal® A81*, a modern aldehyde resin restoration varnish, penetrated though the paint layer preventing the dating of the natural organic binder. Although the wooden panel dating post-dated the artist death and gave a clear sign of forgery, no further evidence could be brought to support the hypothesis that the investigated painting was forged as a result of *Jan Ruyscher*'s rediscovery in the 1930s.[42] The study of an eclectic selection of oil on canvas paintings was met with mitigated results. In two out of three cases, the $^{14}$C age determined from the binding media proved to be a reliable asset in establishing the object's historical context.[43] However, in the third case, the presence of minute amounts of paraffin not identified beforehand compromised the analysis. In another multidisciplinary study of panel paintings from Roman Egypt again the $^{14}$C dating of the binder was offset by several hundreds of years relative to that of the wooden support. The explanation remains unresolved since following the initial sampling the objects, they were fully restored hindering further sampling for identification of additional carbon sources.[44] In another example, a combination of beeswax and gum elemi was found to be the source

of the age bias in the study of the stratigraphy of a polychrome sculpture.[45]

Not only are conservation treatments of concern but very often mixtures between inorganic pigments and synthetic organic lakes occur and even trace amounts may strongly influence the outcome of the results. The latter being exemplified in the analysis of *Hosch*'s paysage (1931), where both the canvas and some green paint were compared (see *Figure 2*).[27] The targeted green was characterized as bearing viridian green, a chromium oxide hydrate pigment, prepared with linseed oil and so suitable for $^{14}$C analysis. The measured $^{14}$C ages of the paint sampled from *Hosch*'s painting was offset by *ca.* 200 years in comparison to the canvas value, which was not anticipated. Complementary spectroscopic analysis identified in the green sample trace amounts of an indamine barium lake (PG9), a modern synthetic dye, which is without any doubt the source of the contamination being a coaltar based synthetic product.

While dating of the binder vehicle in inorganic pigmented paint layer is a relatively new approach in oil painting analysis, it is a much more generalized practice in rock art, where it is the painting/drawing which is sampled provided sufficient organic material is available.[46,47] Nonetheless, the same difficulties as described above are encountered, where despite pigment analysis, anomalous dating results are also gained linked to the presence of exogenous carbon source.[48,49] All of the abovementioned studies are characterized by one common denominator: because the sampled material seldom consists of a unique/single source of carbon, age deviations are frequently observed as it is the bulk's isotopic signature which is
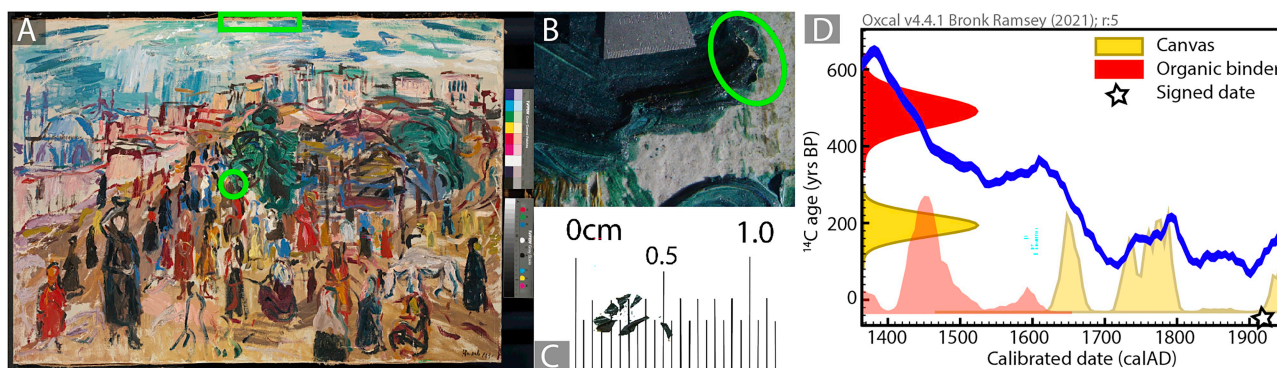


**Figure 2.** A) Stadtansicht mit Figuren in Vordergrund, *Karl Hosch*, 1931, oil on canvas, 70×100 cm, SIK-ISEA, Zurich. Photograph: SIK-ISEA (*Philipp Hitz*). Sampling of the canvas and paint areas are highlighted in green, where the respective damage after sampling is depicted in the close-ups (B) and C) represents the actual sampled paint amount (<1mg paint). D) Comparison of the radiocarbon dating of the canvas and green paint against the Intcal20 calibration curve (in blue), where the paint comes out several hundreds of years older than the canvas. Material originating from *Hendriks* doctoral thesis.[27]

measured. When paint or binding media is mentioned, it encompasses all carbon-bearing materials that are present.

Regarding dating of lead white, the retrieved $^{14}$C ages are generally more reliable in comparison to the binder dates as demonstrated in a study focused on understanding the original polychromy of Portuguese sculptures carved in limestone.[36] The majority of samples confirmed a medieval attribution except for two samples that indicated dates thousands of years old. One had been sampled neighboring a natural blue azurite ($Cu_3(CO_3)_2(OH)_2$) pigment layer, while the second was coated with polyvinyl acetate (PVAc). Both the mineral pigment and the modern restoration adhesive were shown to dissociate/degrade around 300 °C, therefore contributing to the evolved $CO_2$ used in dating, thus biasing the results to older dates.

The diversity of materials in cultural heritage objects represents one of the biggest challenges for $^{14}$C applications, but at the same time this can represent a positive attribute. Many components are organic materials isolated from natural sources and so are chronological markers for the object's history, whether it is in the object's making, use, reception, loss, or restoration. The main weakness of the majority of studies is the failure to address how the $^{14}$C age is estimated based on the average $^{14}$C content of all carbon bearing materials present in the bulk sample rather than targeting a specific organic compound. A physical separation of all different carbon sources has never been achieved or even pursued, only different strategies aiming at the selective removal and/or at the analysis of specific carbon sources have been put forward. While sample size is no longer an issue due to technological advances (AMS, gas ion sources), the sampling location and all the chemistry of the object remains. Thus, it is our task as scientists to ensure that when given the opportunity to sample precious and valuable artifacts, we produce reliable and accurate data rather than excusing inconsistent results with contamination issues. If $^{14}$C dating scientists wish to build a long-lasting relation of trust with the heritage science community, the quality of the data delivered must be unequivocal and indisputable, outweighing by far the necessity of sacrificing a small sample. What is now needed is to account for the various carbon compounds, hereby aiming to isolate single compounds prior to the $^{14}$C dating step, in order to achieve reliable and accurate $^{14}$C dates. Instead of collecting samples from locations based on suitability requirement, a broader choice of sampling zones will be feasible. The separation of the different organic

compounds prior to the dating step will reveal the story of the artwork: when it was produced, when it was altered or restored.

## 2. Is Compound Specific Radiocarbon Analysis (CSRA) the Future of Heritage Science?

The separation of intermingled carbon sources is without question the most difficult problem, yet it is feasible. Indeed, a similar problem was encountered in the dating of environmental matrices (soils, sediments, atmospheric particles) where dating of the bulk material did not reflect the multitude of incoming carbon sources with varying $^{14}$C ages. A solution was conceived in the isolation of selected biomarkers giving rise to compound-specific radiocarbon analysis (CSRA) using preparative gas chromatography (PCGC).[50] Originally developed on *n*-alkanes, *n*-fatty acids and sterols, all sufficiently volatile compounds suitable for a GC approach, liquid chromatography (LC) was rapidly proposed as a preparative alternative, making it possible to examine a wider sample scope extending to nonvolatile compounds as well.[51,52] Nowadays, the technique has evolved and allows the isolation of amino acids[53–57] or chlorophyll photosynthetic pigment[58] within environmental matrices. The expansion of CSRA, which relies on the measurement of 10–100 µg C, has been supported by AMS technological developments, such as the miniaturization of AMS systems (MiCadaS)[59,60] and the capacity to measure even smaller samples, down to micrograms of carbon.[61–64] All of these improvements met the needs of heritage science regarding minimal invasive sampling. Although the method of CSRA was introduced more than two decades ago and represents a more elaborate pre-treatment/purification method of the targeted compounds used for dating, it has not made its way into the field of cultural heritage science. The closest applications are archaeological studies where preserved lipids are extracted from organic residues found in pottery vessels.[65–67] This development revolutionized the field of dietary studies[68] and contributed to a growing interest and recognition of CSRA in geoarchaeology.

### 2.1. Colorants as Targeted Compounds for CSRA in Heritage Science

Throughout the course of history, the artist's color palette has significantly extended and changed, from ground minerals to natural organic pigments and

dyes, to a rainbow of colors synthetically produced. The color of an object provides important information as to its origin, as the comparison of the materials present, the so-called dating materials, with information on their earliest date of discovery, allows to establish the earliest possible date of realization of the object.[69,70] In cultural heritage, the latter is commonly referred to as *terminus post quem*. Being extracted from leaves, bark, roots, flowers, berries, nuts, or even insects or snails, natural organic colorants regroup an extremely varied large class of compounds such as anthraquinones, flavonoids, indigoids, or tannins.[71] Their identification may support geographic and historical appropriateness; but limited chronological information is gained as natural dyes have been used since Antiquity up to today. More precise constraints on the date of creation can be provided by $^{14}$C dating, yet until today no such analysis has ever been conducted. The carbon isotopic ratio of natural organic dyes represents a snapshot of the atmosphere during their year of growth, moreover as these compounds are carbon rich ($>50\%$ C), they represent ideal material for $^{14}$C analysis.

## 2.2. Dye Analysis and Theoretical Basis for CSRA

The application of CSRA in cultural heritage research follows the growing interest in multi-analytical approaches allowing the identification of specific marker components,[72–76] where despite the successful isolation of diverse compounds by chromatographic means no study has investigated their subsequent dating by $^{14}$C analysis. The analysis of natural organic dyes and pigments in cultural heritage objects is challenging in many aspects, including low concentration, susceptible to degradation and presence in complex matrices, which requires highly sensitive analytical tools. While the non-invasive quality of spectroscopic techniques is not to be underestimated,[77,78] the information gained is often distorted, as the organic colorants are frequently found in complexed form with metal ions, either bound to cloth fibers or embedded in paint layers in the form of lake pigments. Chromatographic separation, in contrast, despite requiring sampling, can address this level of complexity, where individuals are separated from their matrix and different components present in a dye mixture may be discriminated.

Dye analysis by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) was pioneered by *Wouters* in the 1980s by first separating red chromophores from the madder plant,[79] then from coccid insects,[80] later indigoids[81] which was followed by the isolation of laccaic acid by *Halpine*.[82] Following these first key publications, progress in selectivity and quality of separation has lead RP-HPLC over the years to established itself as the method of choice for the routine analysis of natural organic dyes.[83–85] Most often coupled to diode array detectors (HPLC–DAD), the additional coupling of a mass spectrometer (MS) to the separation system offers yet another attractive alternative for the analysis of unknown compounds. Based on the fragmentation pattern gained, a chemical structure may be elucidated.[86–88]

Prior the advent of HPLC systems, thin-layer chromatography (TLC) was used for dye analysis and identification.[89,90] Fast, simple, and cheap are its main characteristic. Although it was rapidly supplanted by HPLC system, TLC is nowadays regaining momentum as it is being coupled with surface-enhanced *Raman* spectroscopy (SERS).[91–94] This hybrid technique combining separation techniques and vibrational spectroscopy is specifically tailored to the needs of Heritage science application, regarding both historical textiles and works of art.[95]

Gas Chromatographic (GC) separation of natural organic dyes and pigments has so far found limited application in historical objects, owing to the strong popularity of HPLC. The relatively high molecular mass and polarity of most dyes require derivatization of the targeted analytes into their more volatile counterparts. Although uncommon promising results on GC-MS separation for indigoid dyes and anthraquinoids using acetylation,[96] silylation[97–99] and methylation reagents[100] have been reported. Alternatively, approaches including a pyrolysis step have also been demonstrated with and without prior derivatization.[101–104]

The selected literature review indicates that chromatographic techniques are well established in the analysis of natural organic dyes and pigment, as they play a crucial role in separating the compounds of interest from their heterogeneous matrix. The chromatographic separation is the essence of compound specific radiocarbon dating, where targeted compounds are isolated and purified from the bulk, and hereby the key to producing reliable $^{14}$C dates in the context of heritage science. Although the research questions and specific compounds that are targeted in environmental science and heritage science are quite different, there are many synergies in terms of analytical protocols that may be drawn.

## 2.3. Extraction, Purification of Colorants and Extraneous Carbon Contamination Quantification

The aim of dye analysis is to separate relevant dye markers of a sample to allow an identification, whereas the aim of CSRA is to isolate and purify a reasonable quantity of the selected compound for its subsequent dating by AMS. In its simplistic form the development of a strategy, which will allow to determine a $^{14}$C age on micrograms of a dye molecule, expands on well-established dye analysis protocols already used in the study of cultural heritage artefacts combined with CSRA knowledge in collection and purification of the targeted compounds. Although many parallels may be drawn in terms of analytical steps, in reality, it is much more complex as it requires in-depth understanding of the associated $^{14}$C constraints. The major challenges of the approach are *1)* to separate all interfering carbon compounds in the organic matrix, *2)* to purify adequate quantities of the compound of interest *ca.* 30 µg C, which represents roughly 60–100 µg of colorants as most dyes bear 50–70% C, *3)* to conduct the entire preparation without introducing exogenous sources of carbon contaminants. The latter may have different sources, within the laboratory, from the dye extraction protocol, as well as from the chromatographic separation step, all of which requires a robust assessment.

As summarized by *Ingalls et al.*, one of the key challenges associated with CSRA is to minimize, constrain and effectively monitor the added exogenous carbon ($C_{Ex}$) during the whole sample preparation, chromatographic separation, fraction collection and final AMS measurement (*Eqn. 1*):[105,106]

$$C_{Measured} = C_{Sample} + C_{Ex} \qquad (1)$$

$$\text{Where } C_{Ex} = C_{Chemistry} + C_{matrix} + C_{Chrom} + C_{EA-AMS}$$

where $C_{Sample}$ is the carbon related solely to the sample or compound of interest, $C_{Chemistry}$ is the carbon added through the sample preparation, $C_{matrix}$ relates to carbon impurities that might co-elute with the compound of interest (impurities originating from the matrix), $C_{Chrom}$ the carbon introduced during the chromatographic purification step and finally the combustion step in the EA-AMS, potential cross contamination from the EA column, the zeolite trap, and the AMS syringe is accounted for in $C_{EA-AMS}$. The smaller the samples, the larger the bias, and so assessing the overall level of contamination introduced through the different steps and propagating

the error, is key in producing accurate data and interpretation thereof. Correction strategies leading to the quantification of both the mass $m_{Ex}$ and isotopic ratio (F$^{14}$C$_{Ex}$) of C$_{EX}$, may be evaluated using direct (process blanks) and indirect (2-end-members approach using standards of known $^{14}$C content) methods.[107] While both approaches were shown to agree on the contamination quantification,[106] the direct approach is better suited for contamination > 10 µg C. CSRA applications mandate that contamination levels are below 10 µg C and so the indirect approach is to be preferred. Through mass balance and error propagation, the model of constant contamination assumes that the overall C$_{Ex}$ contribution is constant in both quantity and isotopic ratio, a correction strategy used worldwide to correct CSRA data.[61,63,64,105,106,108–111] The indirect approach requires two standards, one $^{14}$C depleted (F$^{14}$C *ca.* 0) and one $^{14}$C modern (F$^{14}$C *ca.* 1). While this approach is more labor intensive as it requires the set of standards to be processes throughout the whole sample preparation, it enables to estimate not only the overall level of contamination C$_{Ex}$, but more precisely $^{14}$C enriched or fossil contaminations. Unknown samples are consequently corrected for C$_{Ex}$ based on the mass-balance correction expressed in *Eqn. 2*:

$$F^{14}C_S = \frac{F^{14}C_m \cdot m_m - F^{14}C_{Ex} \cdot m_{Ex}}{m_m - m_{Ex}} \qquad (2)$$

where $m_m$ denotes the total measured C mass, which is the sum of the actual C mass of the sample $m_S$ and of the contamination $m_{Ex}$. Correspondingly, the measured $^{14}$C content is given by F$^{14}$C$_m$, F$^{14}$C$_S$ represents the sample's $^{14}$C content and F$^{14}$C$_{Ex}$ the one of the contamination. The corresponding uncertainty is thereafter derived from error propagation. Here, not only will the methods benefit from technological advances in AMS, but the acute monitoring of the added exogenous carbon is supported by improved results on blank assessments and constant contamination correction strategies published by many different research teams around the world.

## 3. From Theory to the Laboratory Bench

The proposed methodology is subdivided into three main steps: *1)* Adapted chemical extraction of natural organic dye and pigment, *2)* selection of a suitable chromatographic separation technique, and *3)* final $^{14}$C analysis. The following section contains discussion and

rationale regarding preliminary results in view of demonstrating the potential impact of CSRA in heritage science with an emphasis on the selection of a suitable chromatographic separation technique and monitoring of the exogenous ^{14}C sources (see *Figure 3*). With the range of natural organic dyes and possible matrices being extremely diverse, the proof of principle experiment scope was narrowed to dyed textiles, *i.e.*, with natural fibers as substrate, and a single dye with the isolation of alizarin, a red chromophore of the anthraquinone family.

### 3.1. Adapted Chemical Extraction

In traditional dye analysis, an extraction is necessary to separate the dye or pigment from its organic matrix, whether textile fibers or artist's paints. Owing to the variety of chromophores, which may be sorted based on their color properties, their biological source, their method of application or chemical structures, no holistic extraction method is possible. Three standard extractions protocols may however be distinguished: acid hydrolysis in a methanolic solution, use of complexation agents such as oxalic acid or EDTA, or use of organic solvents such as pyridine, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF).[112] While all involve the use of carbon-bearing chemicals, the acid hydrolysis shows the most promise for downstream ^{14}C application. The dipolar aprotic solvents

(DMSO, DMF) and pyridine are rather sticky, with boiling points $> 100\,°C$ and susceptible to cause residual solvent contamination. In contrast, in the traditional approach, known as the *Wouter*'s method, the sample is treated in a solution of water/MeOH/ 37% HCl (1:1:2, *v/v/v*) for 10 min at 100 °C, which implies the use of hydrochloric acid a non-carbonaceous acid while MeOH readily evaporates owing to its low boiling point. A potential problem of this acid protocol is the degradation of acid-labile glycosylated chromophores.[113] Furthermore, the harsh acidic conditions tend to depolymerize protein-based fibers, adding another degree of difficulty to the separation process.[114]

### 3.2. Chromatographic Technique Selection

All chromatographic techniques carry the potential for coupling to ^{14}C analysis, however they are also known to introduce carbon contamination, through the column bleed, incomplete solvent removal and coelution of compounds. While HPLC has established itself as the method of choice for the routine analysis of natural organic dyes supplanting TLC and GC, both retained attractivity with regard to ^{14}C constraints. Indeed, the typical reported chromatographic procedural blanks for HPLC are in the order of 5 µg C,[53,54,57,115] whereas preparative GC was shown to achieve lower column bleed extraneous-carbon
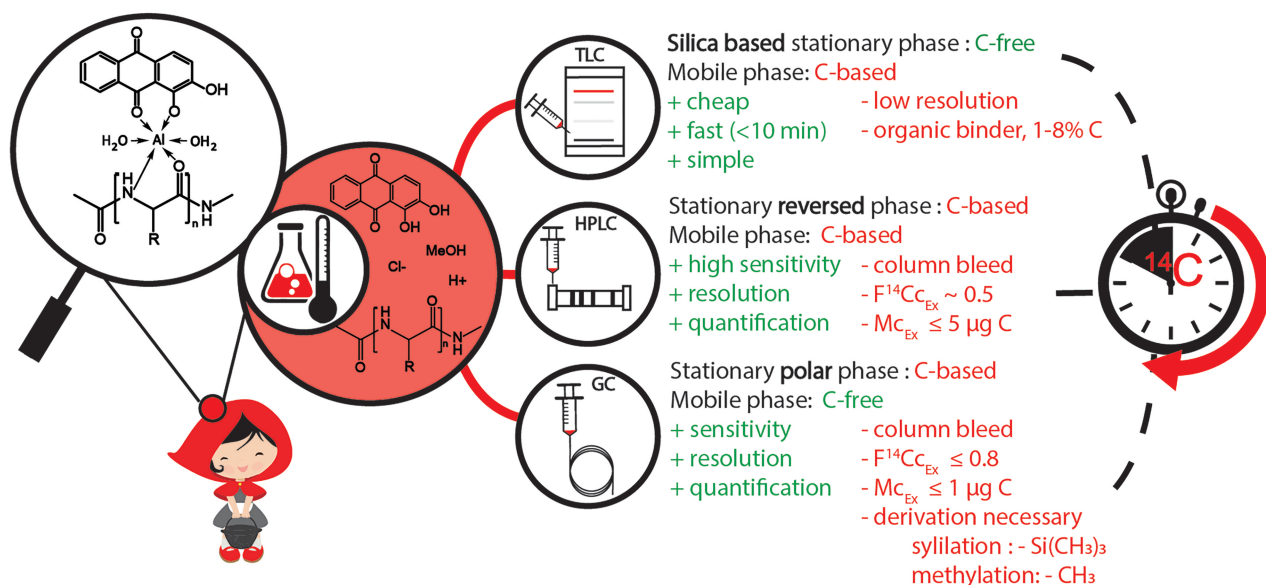


**Figure 3.** Schematic diagram illustrating the different steps required to obtain compound-specific ^{14}C results from red dyed textiles, from extraction of alizarin of the matrix and isolation through chromatographic means and final ^{14}C analysis by AMS. The given mass and isotopic value of $C_{Ex}$ are based on averaged reported values in the literature.

contamination.[62,116,117] Silica based TLC was considered, as a potential alternative as in theory the stationary phase is carbon free. In practice, however, for the coating to adhere to the plate a small amount of binder is necessary, which is generally based on organic material. Elemental analysis of various TLC plates featured between 1–8% carbon, which when ^{14}C dated appeared to carry an isotopically depleted ^{14}C signature (F[14]C = 0) and $m_{Ex} > 20$ μg C after elution.

Although preparative GC was shown to achieve lower extraneous-carbon contamination than HPLC in the literature with less than 1 μg C, the need for derivatization to convert nonvolatile organic dyes into a GC amenable derivative represents a non-negligible source of exogenous carbon. While a comparative study between HPLC and GC identification of anthraquinones constituents derived from madder roots showed no clear advantage of one method over the other,[97] preliminary studies showed that attaining full derivatization of anthraquinones was not trivial. In consideration of the downstream ^{14}C coupling, the methylation reaction is advantageous as it introduces only one exogenous carbon per hydroxy function, while the trimethylsilyl derivatives bear three C-atoms. While a mass balance allows to correct for the contribution of the added derivatizing carbons,[65,118] the magnitude of the total propagated uncertainty was deemed to blow up the final error on the measurement and hereby compromising the necessary accuracy and precision demanded for heritage studies. Hence, HPLC was selected as most promising candidate for coupling with ^{14}C analysis.

### 3.3. Alizarin Specific Radiocarbon Analysis: Preliminary Results

For the blank assessment of radiocarbon analysis on red dyes of the anthraquinone family, alizarin ($C_{14}H_8O_4$, MW = 240.21 g/mol) from grinded madder roots *Rubia Tinctoria* L. harvested in 2018 (F[14]C = 1.0042 ± 0.001) was used as modern standard. Its synthetic counterpart was purchased from *ACROS ORGANICS*, purity 97% (F[14]C = 0.0001 ± 0.0001) and used as fossil standard. The separation was carried out on a *1260* series HPLC system (*Agilent*, Santa Clara, USA) with DAD detector, monitored at 280 and 430 nm, using a *Zorbax Eclipse C18*, 4.6 × 100 mm, 3.5 μm column. The compounds were eluted by a binary system of solvents, A = *MilliQ* water – 0.1% phosphoric acid (85 wt-% in $H_2O$, *Merck*, Darmstadt, Germany) and B = MeOH (*Optima*, LC–MS grade, *Fischer scientific*). A linear gradient beginning at 60%

B, then increasing to 90% over 6.5 min, a fast increase to 100%, hold for 3 min, before recovery of the initial conditions over 0.5 min and equilibration over 5.5 min. The injection volume was 10 μL, and *ca.* 10–25 repeated injections were conducted to collect sufficient mass of individual standard approximately *ca.* 5–30 μg C. Using the direct approach, injection blanks on the HPLC lead to an estimation of $C_{Chrom}$, *i.e.*, the column bleed, of 5 μg C and thus necessitating a post-chromatography purification step. Following current trends in literature, elution through 1 cm precombusted silica gel in a glass pipet was found to be the most effective. Although this approach shows promise in removing the column bleed, it still requires optimization, as the samples recoveries vary between 50 to 95%. The samples were then measured at the Laboratory for Ion Beam Physics at ETH Zurich on an AMS (MICADAS) system equipped with a gas ion source (GIS). The constant contamination parameters for $C_{Ex}$, derived from the mass-balance correction represented by *Eqn. 2*, were found to be 3.1 ± 0.8 μg C with a F[14]C$_{Ex}$ of 0.45 ± 0.07. From here, one can increase the complexity and assess the extraction of dyed textile, where the $C_{chemistry}$ contribution must be evaluated. Considering that the dye must first be separated from its matrix, an acid hydrolysis, specific to break the mordant-dye bond, was performed on wool yarns previously dyed with the given set of standards. An additional carbon source $C_{matrix}$ coming from the substrate itself must be considered as proteinous fibers are known to hydrolyze under strong acidic conditions and so increase the baseline (see *Figure 4*), which also contributes to $C_{Ex}$. When comparing process blanks, *i.e.*, undyed wool yarns ($C_{Matrix + Chemistry + Chrom + EA-AMS}$) and direct blanks on the HPLC ($C_{Chrom + EA-AMS}$), both yielded values of the same magnitude indicating that $C_{Matrix + Chemistry}$ contribution is minor if not negligible with respect to the chromatographic and AMS step $C_{Chrom} + E_{A-AMS}$.

## 4. Synthesis and Outlook

While ^{14}C dating is without a doubt a powerful tool to help answer time-related questions, the challenge remains in the choice of the targeted material, the sample selection and characterization. Recent developments in microsamples and CSRA may prove revolutionary in the field of heritage sciences, where the complex nature of the samples and the sampling limitations will be addressed by innovative, inter- and multidisciplinary approaches. The wide spectrum of
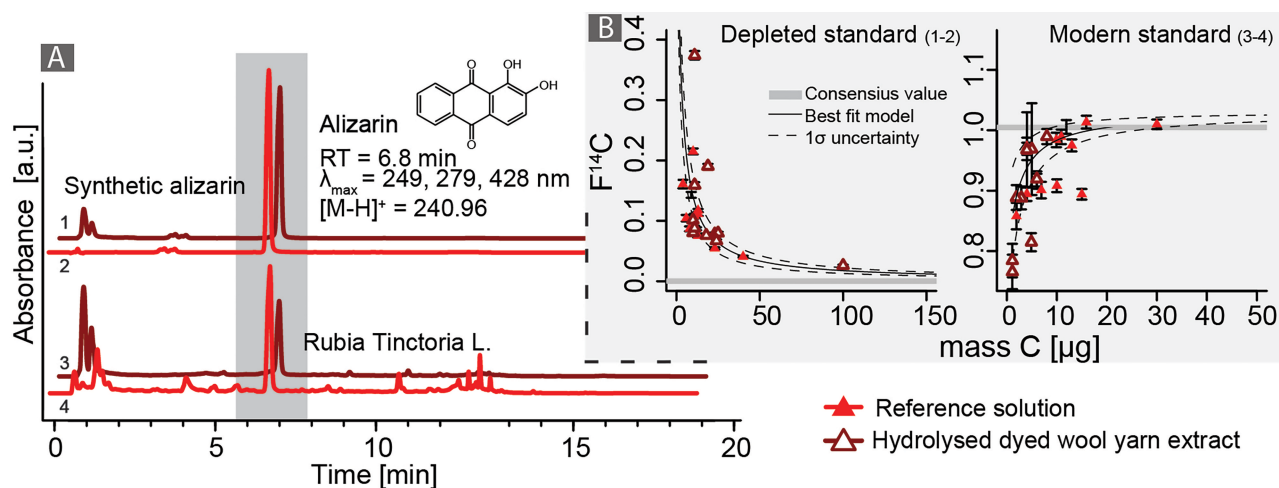
**Figure 4.** A) HPLC chromatogram at 280 nm of a hydrolyzed extract of wool yarns dyed with synthetic alizarin (*1*) and compared to a reference solution prepared with synthetic alizarin (*2*), respectively hydrolyzed extract of wool yarns dyed with madder roots *Rubia tinctoria* L. (*3*) in comparison to a MeOH crude extract of *Rubia tinctoria* L. (*4*). Interestingly, while the madder roots contain over 35 anthraquinone derivatives, only few are transferred to the dyed textile. In both wool extracts, the first two peaks eluting within the first minutes of the chromatogram are assigned to wool degradation products. B) Constant contamination model for HPLC method for isolation of alizarin, for fossil (left) and modern (right) standard. The red filled triangles represent data collected from the injection of stock solutions, whereas the empty triangles represent dyestuff isolated from dyed wool yarns following acid hydrolysis. The solid curve represents the best fit and the dashed lines the corresponding 1σ uncertainty.

material and conservation practice found in artworks and museum objects is one of the biggest challenges for radiocarbon dating, where natural organic dyes and pigments appear as a promising dating proxy. The subtlety in their $^{14}$C dating resides in their chromatographic separation, isolation and purification from complex organic matrix carrier. The described project relies on the effective collaboration between science and art partners. Indeed, very often precise queries from fields of applied science are the drive to technological developments, as pointed out by *Leona et al.* in 2021: '*the analytical challenges posed by studying artists' materials and techniques can stimulate scientific discoveries*'.[119]

Together with other disciplines involved in heritage science, the proposed methodology will offer a deeper insight to the technology of manufacturing, trade routes and commercial transactions that may have allowed the use of certain dyes far from their native locations. Resources available during particular periods and geographical areas will be revealed, providing valuable data about the lifestyle and the technical knowledge of a given population in a defined period, triggering discussions beyond the scientific contribution, generating interest with art experts, historians and the general public.

Nonetheless, although the provided preliminary results support the conceptual premise that such an

approach is feasible many questions arise. The complexity in isolating the dye pigment in conjunction with multiple exogenous carbon sources has important implications for developing a suitable and robust CSRA strategy, thus exploring other chromophores as well as increasing the substrate complexity must all be considered in the methodological development. At this point, matching the C to be analyzed from heterogenous complex artwork with the research question is the biggest challenge. Minimizing the added carbon contamination and quantification thereof, as well as increasing the recovery and purification of the individual compounds, will be necessary to produce true $^{14}$C ages which may be appropriately interpreted. At present, all aspects require further investigation. The authors will report on their progress in future contributions.

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## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author Contribution Statement

*L. H.* and *C. P.* designed the research; *L. H.* performed and analyzed the experiments; *L. H.* and *C. P.* wrote the manuscript.

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