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Linking historical recipes and ageing mechanisms: the issue of 19th century iron gall inks

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ABSTRACT

Several manuscripts and drawings of our historical and artistic heritage have been produced with iron gall inks. To obtain an iron gall ink, ancient treatises cite the addition of ferrous sulphate and gum Arabic to a decoction of oak galls as a basic procedure. Owing to the development of synthetic chemistry, iron gall ink recipes were improved with new materials and procedures in the late 19th and early 20th century. Notably, many conservation issues arise from the interaction between iron gall inks and the paper support of manuscripts and drawings. To date, most of the research on the topic are focused on paper preservation by non-destructive analytical methods, which provide only limited information on degradation process trends and minor components, representative of iron gall ink's recipes. In the present work, three historical recipes of iron gall inks (*alizarine ink, Reid ink, modern gall ink)*, dated to 19th-20th century and differing for the preparation method and additives, were characterized. The molecular markers of iron gall inks and of gallic acid degradation were detected by an optimized protocol based on high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC–HRMS). Furthermore, by performing ageing tests on reference materials in different indoor conditions (natural light and stored in the dark), two degradation mechanisms were observed: hydrolysis of poly-galloyl glucose species and auto-oxidation of gallic acid. Thus, different chemical profiles and ageing trends were revealed depending on the starting recipe. The procedure Limit of Detection (LOD) was estimated, improving the approaches reported so far in the literature. Finally, the strategy was successfully applied for the characterisation of the ink employed in a very degraded 16th century manuscript, granting access to the complete molecular profile of an iron gall ink with just 15 μg of sample.

Introduction

In the history of European civilization, the application of iron gall inks for writing and drawing has been handed down from the 12th to the 20th century, and they are amongst the most commonly employed in the historical and artistic heritage [1]. These writing materials have fascinated generations of men over the centuries [2], leading to the production of large numbers of iron gall containing documents, such as manuscripts, letters, and drawings [3]. Several iron gall ink recipes are reported in ancient treatises [4–6], along with their evolutions over the centuries. However, regardless of the century of production, most of the recipes mention the use of a decoction of gallnuts in combination with iron (II) sulphate and gum Arabic for a black ink. For preparing an iron gall ink, two main steps were required. First, crushed gallnuts were treated with several solvents (e.g. boiling water, wine, alcohols) to promote the extraction of *gallotannins* [7], or at least of the most abundant hydrolysable polyphenols fraction in gallnuts [1]. This class of compounds consists of poly-galloyl glucose (i.e., poly-galloyl esters of glucose) and poly-galloyl gallates (i.e., poly-galloyl esters of gallic acid). Their molecular structure is reported in Fig. 1. Second, iron (II) sulphate was added to promote the formation of iron-polyphenols complexes. Both poly-galloyl glucoses and gallates played a fundamental role in the complexation of Fe^{2+} ions [3], leading to the formation of Fe^{3+}- polyphenols complexes responsible for the deep black colour of iron gall inks [8]. To promote the oxidation of Fe^{2+}- polyphenols to Fe^{3+}- polyphe-

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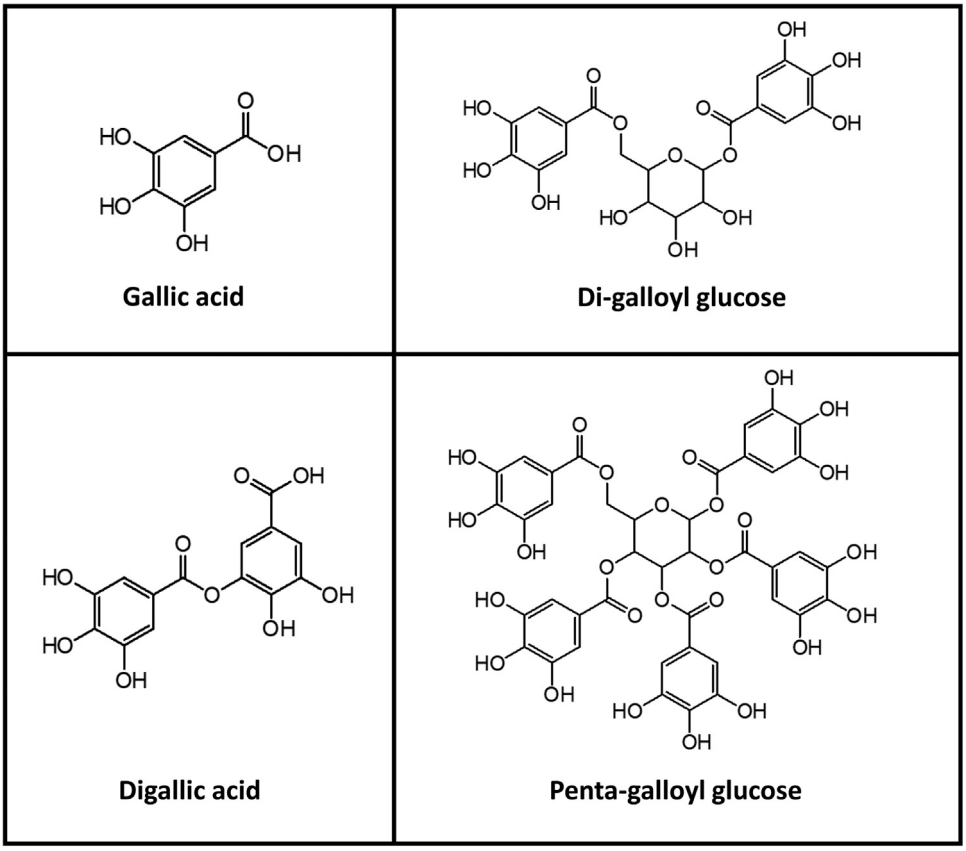


Fig. 1. Molecular structure of gallic acid ($C_7H_6O_5$), di-galloyl glucose ($C_{20}H_{20}O_{14}$), digallic acid ($C_{14}H_{10}O_9$), and penta-galloyl glucose ($C_{41}H_{32}O_{26}$).

nols complexes, the extract is exposed to atmospheric oxygen for a different period depending on the traditional recipes.

In the 19th century, driven by industrial developments and synthetic chemistry, iron gall ink formulations drastically changed [9]. New ingredients and procedures were introduced to improve the writing performance of the inks, developing different options: "traditional", "oxidised", and "unoxidised" iron gall inks [4,10]. Moreover, the use of free acids, metallic iron, and interim dyes (e.g., indigo, indigo carmine, and aniline colourants) became more and more frequent, yielding inks with greater penetrating power, and more resistance to bleaching agents, but also with a higher corrosive power. Indeed, iron gall inks are not only famous for their deep black colour and wide usage, but also for many issues related to iron gall ink fading [11–13], and to the conservation of the support, such as paper ink-induced corrosion [14–18]. In addition, the chemical analysis of writing inks faces several challenging analytical issues, such as the variety of materials and recipes used over the centuries, the complicated interactions between paper and the metal-ink complex, the depth of penetration of the writing lines, and the few studies available in the literature on degradation processes occurring on the dyes constituting the inks. The difficulty in sampling, combined with the fragility of the inked works has promoted the application and development of non-destructive techniques, such as Raman [19–21], X-Ray Fluorescence [22–24] and Fiber Optics reflectance spectroscopies [25–27], as recently reviewed by Melo et al. [8] and Caterino et al. [1]. However, these techniques provide limited information on degradation products and minor components whose detection can be useful for dating and authentication purposes. Furthermore, the complexity of the chemical profile associated to tannin-based inks makes the application of chromatographic techniques fundamental to thoroughly determine their composition [3,28].

Research aim

To improve our global knowledge on paper corrosion issues, in the present work different historical iron gall ink recipes were studied. Our research aim was the investigation of iron gall ink ageing mechanism that may involve the organic component, reported so far in the literature only regarding the gallic acid [10]. A protocol based on liquid-chromatography coupled to tandem mass spectrometry (HPLC-ESI-Q-ToF) and optimized for a model iron gall ink [10], has been applied for the study of two 19th –20th century historical recipes found in a 1904 compendium [4] and one simplified reconstruction [20]: *alizarine ink, Reid ink,* and *modern gall ink*. The analytical strategy proved to be effective both for highlighting the differences in iron gall ink recipes and for studying ageing processes. Moreover, to support the restoration and conservation tasks, different ageing conditions were tested to determine the influence of environmental parameters. The two different indoor conditions applied (natural light and stored in the dark) allowed us to outline two degradation mechanisms: hydrolysis of poly-galloyl glucose species [7] and auto-oxidation of gallic acid to ellagic acid [10]. Different rates of ageing were observed based on iron gall ink recipes, in association with the production of degradation markers partially reported so far in the literature.

Materials and methods

Chemicals

Gallic acid and ellagic acid were purchased from LabService Analytica (Italy) and Lancaster (UK), respectively. Standard solutions were prepared in dimethyl sulfoxide (DMSO; 99.8% purity; J.T. Baker, USA). The solvents used for HPLC-ESI-Q-ToF analysis and

sample pre-treatment were: water (LC-MS grade, Sigma Aldrich, USA), acetonitrile (LC-MS grade, Sigma Aldrich, USA), formic acid (98–100%, Sigma Aldrich, USA), ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich, USA) and N,N-dimethylformamide (Sigma Aldrich, USA). The reagents and materials used for preparing the reference inks were: oak galls (Kremer Pigmente, Germany), ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$; Analyticals, Carlo Erba, Italy), chippings of metallic iron (Carlo Erba, Italy), gum Arabic (Kremer pigmente, Germany), indigo (kindly provided by the Textile Sector of the *Opificio delle Pietre Dure*, OPD, Florence), glacial acetic acid (99–100% purity, J.T. Baker), and deionized water.

Reference inks and mock-ups

In the present work three historical iron gall ink's recipes were studied: *alizarine ink* [4], *Reid ink* [4], and *modern galls ink* [20]. The modern galls ink was prepared using a simplified procedure [20], which is adapted from historical recipes, while alizarine and Reid inks were prepared accordingly to historical formulations described by Leonhardi (1855) and Reid (1827), respectively [4]. The amounts of materials and water were scaled down, keeping unaltered the relative ratios of the ingredients:

- *Alizarine ink*: 50 mL of decoction of oak galls (prepared as reported in Supplementary Information file, S1.1) were added with 2.3 g of metallic iron, 3.0 mL of glacial acetic acid, 5.3 g of ferrous sulphate and 1.3 g of indigo.
- *Reid ink*: 42 mL of decoction of oak galls (prepared as reported in Supplementary Information, S1.1) were mixed with 8.9 g of ferrous sulphate and 8.9 g of gum Arabic. The mixture was exposed to air at room temperature for one day, and then filtered.
- *Modern galls ink*: 1.3 g of crushed oak galls were boiled for 30 min in 60 mL of deionised water. Then, 1.1 g of ferrous sulphate was solubilized in 5 mL of water. The two solutions were mixed, and the resulting solution was exposed to air at room temperature for one week. Finally, 1.6 g of gum Arabic were added, and the resulting ink was filtered to remove any solid residues.

Reference ink mock-ups were prepared casting the ink replicas on paper supports (Whatman filter paper, USA, grade 42, diameter 110 mm, pure cellulose), according to [10]. Ageing tests were carried out in two different indoor ageing conditions (T = 25–27 °C, RH c.a 50% [10]): exposed to natural light through a glass window and stored in the dark. For the analysis, samples were collected by a micro-hole hand-held paper puncher in different points of the surface to correct for the variability due to the inhomogeneity of the casting.

Liquid chromatography – tandem mass spectrometry

HPLC-ESI-Q-ToF analyses were carried out according to an optimized protocol for the separation and detection of the organic dyes in iron gall inks [10]. An HPLC 1200 Infinity, coupled to a Jet Stream ESI-Q-ToF 6530 Infinity detector and equipped with an Agilent Infinity autosampler (Agilent Technologies, Palo Alto, CA, USA) were used. The chromatographic separation was performed on an analytical reversed-phase column Poroshell 120 EC–C18 (3.0 × 75 mm, particle size 2.7 µm) equipped with two different guard-columns: a) Zorbax guard-column (4.6 × 12.5 mm, particle size 5.0 µm) b) InfinityLab Poroshell 120 EC–C18 guard-column (3.0 × 5.0 mm, particle size 2.7 µm). Both column and guard-columns used were Agilent Technologies (Palo Alto, CA, USA). During the first year of measurements, guard column (a) was chosen even if sub-optimal, and guard-column (b) was introduced as soon as core-shell precolumn of a suitable size became commercially available. Specifically, the chromatographic set-up described in a) was used for the analysis of unaged, aged at natural light for six and twelve months, and aged at dark for twelve months reference mock-ups, while the set-up reported in b) was employed for the analysis of Warsaw manuscript, and both aged at natural light and at dark for twenty-four months reference mock-ups. Prior to liquid chromatography analysis, both reference ink mock-ups and the sample from the Warsaw manuscript were treated with 0.1% of EDTA in H_2O/DMF 1:1. Then, the extract was placed in an ultrasonic bath for 1 h at 60 °C, the supernatant filtered with PTFE syringe filters (4 mm thickness and 0.45 µm pore diameter, Agilent). Different extraction volumes were used depending on sample size. For the ageing study, 500 µL of extraction solution were used, whereas smaller volumes were applied for the historical sample (see below). Finally, 4 µL (reference ink mock-ups and Warsaw manuscript 3 mg) or 15 µL (Warsaw manuscript 15 µg) were injected in the chromatographic system. Further details regarding the analytical parameters and working condition can be found in [10].

Semi-quantitative analysis

To monitor the ageing of the iron gall ink mock-ups, data based on peak areas obtained for the Extract Ion Chromatograms (EICs) were normalized to 100 and the data matrix obtained was used for plotting representative histograms. Reproducibility of polyphenol area was calculated analysing Reid ink mock-up aged for six months at natural light in triplicate. The coefficient of variation (CV%) was estimated for gallic acid, ellagic acid, poly-galloyl glucose and poly-galloyl gallates and reported in Table S.1. A CV% lower than 4% was obtained for all the polyphenolic compounds.

Quantitative analysis

Stock and standard solutions (0.5, 1.0, 2.5, 5.0 and 10 ppm) of gallic acid and ellagic acid were prepared in DMSO and each concentration level analysed in triplicate. The calibration curves were obtained by integrating the Extract Ion Chromatograms (EICs) peak areas of gallic and ellagic acid. Limit of detection (LOD) and limit of quantification (LOQ) were estimated using the standard solution with the lowest concentration level giving a visible signal and the calibration curve with $q = 0$. The linear regression fitting parameters, LODs and LOQs obtained are reported in Table S.2. The instrumental LODs estimated for gallic acid and ellagic acid considering the procedure with 500 µL of extraction solution are c.a. 18 ng and 12 ng for gallic acid and ellagic acid, respectively.

To estimate the procedure LODs and the minimum amount of sample required for the analysis, artificial aged (RH 30%, four weeks [10]) alizarine ink mock-up, suitable as proxy for an unknown sample, was used to simulate ageing conditions more demanding than those employed in the present study. The reference mock-up was sampled on the surface by a scalpel. Different amounts of sample were tested and treated with reduced volume of 0.1% EDTA aqueous solution/DMF: **a)** 30 µg of reference ink mock-up in 150 µL; **b)** 100 µg of reference ink mock-up in 150 µL; **c)** 500 µg of reference ink mock-up in 200 µL; **d)** 800 µg of reference ink mock-up in 200 µL of extracting solution. The HPLC-ESI-Q-ToF analysis of **b)** were repeated in triplicates, and the data acquired were used to calculate the LOD of procedure. The LOD values calculated are reported in Table 1:

Historical ink sample

In this work, samples collected amongst fragments detached from a 16th century Polish manuscript (private collection of Dr. Barbara Wagner, University of Warsaw) were analysed. Two fragments of different weight were analysed: a bigger one was sacrificed to clearly outline the degradation pattern (3 mg extracted in

Table 1
LOD of procedure calculate for gallic acid and ellagic acid by analysing 100 µg of reference ink mock-up in 150 µL of extract. For each compound the mean value of concentration (C), the standard deviation (S), the coefficient of variation (CV%), the limit of detection on the extract ($LOD_{extract}$) and the limit of detection in terms of analyte weight in the sample ($LOD_{procedure}$) are reported.

	C (ppm)	S (ppm)	CV%	$LOD_{extract}$ (ppm)	$LOD_{procedure}$ (ng)
Gallic acid	5.25	0.04	1	0.12	32
Ellagic acid	0.57	0.03	5	0.09	23

500 µL) and a smaller one to check whether we could obtain significant results with samples even smaller than those tested for the assessment of the LOD of procedure (15 µg in 50 µL of extracting solution).

Results and discussion

The iron gall inks investigated in this work were prepared according to historical ink recipes (*alizarine* and *Reid ink*) or to a modern recipe inspired by historical ones (*modern galls ink*). Reid and modern gall inks both entail the two basic steps (i.e., addition of iron (II) sulphate hepta-hydrate and gum Arabic to a gall's decoction) followed by a period of exposure to air, and then filtration. Specifically, the two formulations differ in the time of exposure to atmospheric air (one day for Reid ink, and one week for modern galls ink) prior to filtration. Differently, for *alizarine ink* preparation, metallic iron (Fe^0) and acetic acid were used as additives in its formulation, and exposure to atmospheric air and the use of gum Arabic are not reported. According to the literature [4], following this recipe prevents the oxidation of the Fe (II) - polyphenols complexes [28], and provides the resulting ink with a greater penetration power into the paper support than traditional iron gall inks (e.g., Reid and modern galls inks). *Alizarin ink* is thus classified as "unoxidised iron gall ink".

Unaged reference mock-ups

HPLC-ESI-Q-Tof analysis allowed us to outline the molecular profile of the EDTA-DMF extracts of the mock-ups of alizarine, Reid, and modern galls, both before and after ageing. The Extract Ion Chromatograms (EICs) of the EDTA-DMF extracts of unaged ink mock-ups are reported in Fig. 2, while the detected compounds are listed in Table S.3. Gallic acid, ellagic acid, poly-galloyl glucose species (mono-, di-, tri-, tetra-, penta-, hexa- and hepta-galloyl glucose) and poly-galloyl gallate species (digallic and trigallic acid) were detected as main molecular markers in the three inks. Moreover, HPLC-ESI-Q-ToF analysis of unaged mock-ups enabled us to outline the following differences between the formulations:

- Qualitatively, *Reid* and *modern gall inks* show similar polyphenolic profiles, also featuring several gallic acid degradation products [10] as secondary components (Figure S.1, a). Since these compounds have not been identified in *alizarine ink*, their formation can be ascribed to processes occurring during the exposure to atmospheric air;
- Ellagic acid was detected in relatively high amounts in *Reid* and *modern gall inks* while it was identified at traces level only in the unaged *alizarine ink* mock-up (Fig. 2). This could prove that the presence of metallic iron in *alizarine ink* formulation inhibits not only the oxidation process of Fe (II) - polyphenols complexes [28], but also the auto-oxidation of gallic acid during ink preparation [10]. Besides ellagic acid, also poly-galloyl gallates are known intermediates of auto-oxidation processes [10]; however, they are already present in non-negligible amounts in the oak gall decoction used for the preparation of the ink;

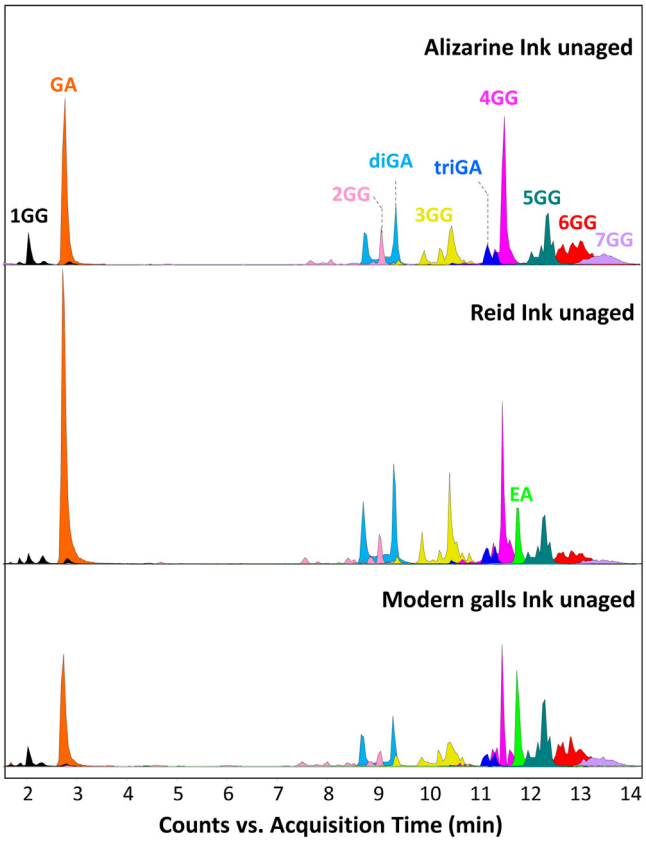


Fig. 2. HPLC-ESI-Q-ToF Extract Ion Chromatograms (EIC) of polyphenolic compounds detected in unaged Reid, alizarine, and modern galls ink mock-ups. Negative acquisition mode. Extract Ion Chromatograms (EIC) of the main components identified: $C_7H_6O_5$ (gallic acid, GA), $C_{14}H_6O_8$ (ellagic acid, EA), $C_{14}H_{10}O_9$ (digallic acid, diGA), $C_{21}H_{14}O_{13}$ (trigallic acid, triGA), $C_{13}H_{16}O_{10}$ (mono galloyl-glucose, 1GG), $C_{20}H_{20}O_{14}$ (di galloyl-glucose, 2GG), $C_{27}H_{24}O_{18}$ (tri galloyl-glucose, 3GG), $C_{34}H_{28}O_{22}$ (tetra galloyl-glucose, 4GG), $C_{41}H_{32}O_{26}$ (penta galloyl-glucose, 5GG), $C_{48}H_{36}O_{30}$ (hexa galloyl-glucose, 6GG), and $C_{55}H_{40}O_{34}$ (hepta galloyl-glucose, 7GG). All chromatograms are presented in the same scale and are stacked for purpose of clarity.

- Semi-quantitative differences were observed when comparing unaged ink profiles pointing at as different ingredients and steps significantly influence the molecular composition (Figure S.2). This result is in agreement with the paper of Teixeira et al. [3], who detected the same polyphenols in Iberian iron gall ink's recipes, whose profiles are recipe-dependent.

Aged reference mock-ups

We applied the analytical protocol to the reference ink mock-ups. The results enabled us to compare the profiles obtained for the aged samples to the profile of the corresponding unaged ink, and to highlight effects related to the use of different recipes. By studying the profiles corresponding to ageing performed by indoor tests at natural light and in the dark, both qualitative and semi-

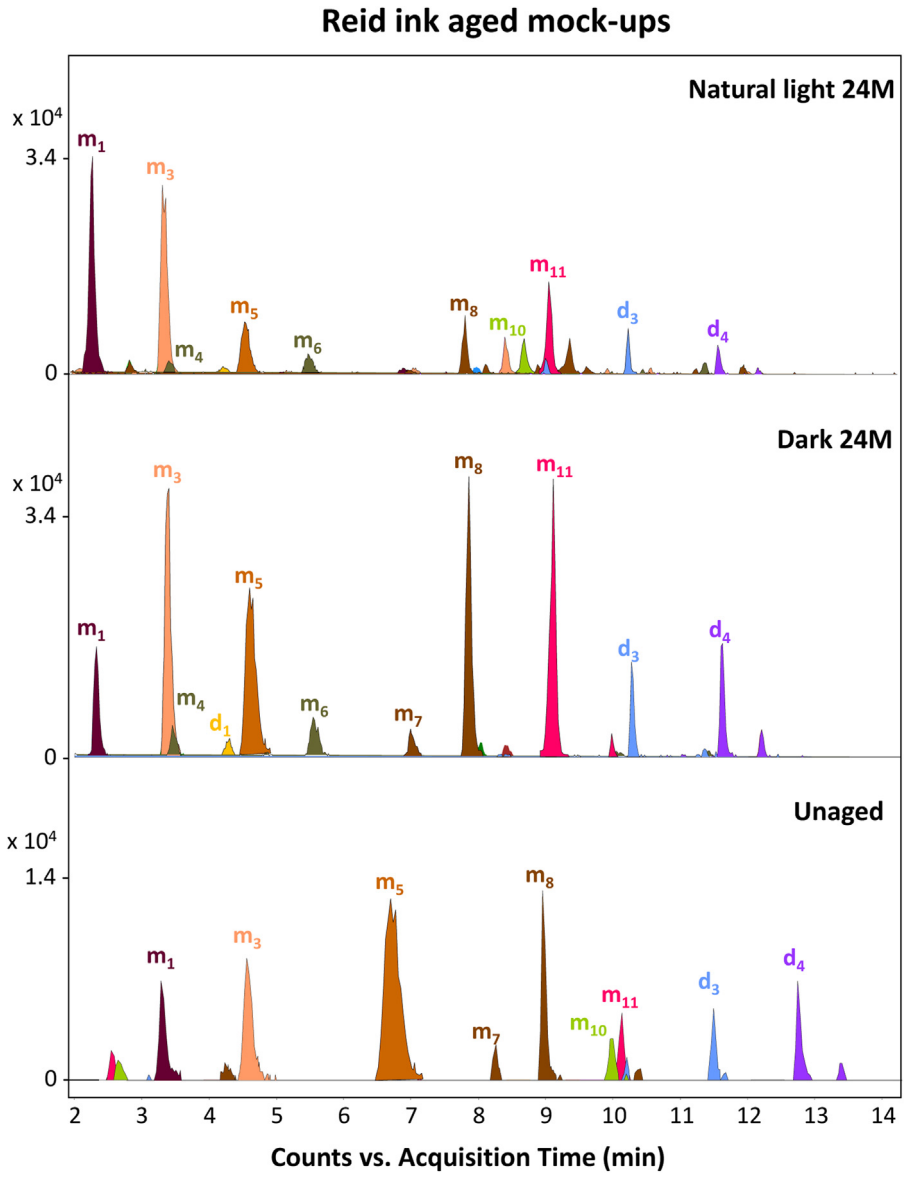


Fig. 3. HPLC-ESI-Q-ToF Extract Ion Chromatograms (EIC) of Reid ink degradation products: $C_8H_6O_7$ (m_1), $C_{10}H_6O_6$ (m_2,m_7,m_8), $C_8H_6O_6$ (m_3), $C_9H_8O_6$ (m_4,m_6), $C_8H_6O_5$ (m_5), $C_9H_8O_5$ (m_{10}), $C_{10}H_8O_7$ (m_{11}), $C_7H_6O_4$ (d_1), $C_{12}H_8O_6$(d_2, d_3), and $C_{13}H_8O_6$ (d_4) from the EDTA-DMF extract of mock-ups unaged, and aged for twenty-four months at dark or to natural light. Negative acquisition mode. The EICs showed in a were acquired with Zorbax guard-column; instead, for b and c InfinityLab Poroshell 120 EC–C18 guard-column was used.

quantitative differences were observed. All HPLC-ESI-Q-ToF Extract Ion Chromatograms (EIC) of *alizarine, Reid*, and *modern galls* inks degradation products are provided in Figure S.1, while in Fig. 3 representative molecular profiles of unaged and aged Reid ink are reported.

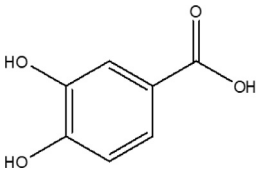
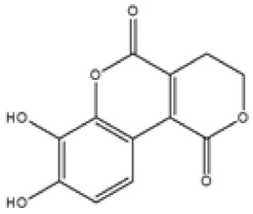
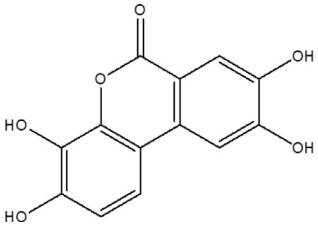
Qualitative analysis

The qualitative analysis allowed us to ascertain that ageing processes promote the production of ageing and/or degradation markers. Some of these markers were already determined in the corresponding unaged reference mock-ups of *Reid* and *modern galls* inks, and their formation could be associated to the oxidation process induced by the exposure of the ink to air. Nonetheless, as ageing progresses, changes in their relative amounts were observed, proving that the formation of these markers is promoted during the ageing mechanisms of iron gall inks. The EIC profiles obtained are reported in Fig. 3 and Figure S.1. In addition to the gallic acid degradation markers already described in [10] and deriving from gallic acid, further ageing products were determined. The MS and MS^2 results (Table 2 and Figures S.3-S.4) enabled us to characterise these compounds as 3,4-dihydroxy benzoic acid (d_1, t_R = 4.2 min, confirmed by comparison with an analytical standard), urolithin D (d_4, t_R = 11.5 min) and two isomers of an unknown ageing product with a pseudo-molecular ion of $[M\text{-}H]^-$ = 247.025 (d_2, t_R = 8.9 min; d_3, t_R = 10.2 min). Since these markers have not been determined in a model gallic acid ink [10], their formation could not be associated directly to the degradation of gallic acid. However, they can be originated by the degradation process undergone by ellagic acid, poly-galloyl glucose or gallate, not yet investigated by ultra-sensitive techniques, or by the depolymerisation of condensed tannins, which cannot be detected by HPLC-ESI-Q-ToF given their insolubility in the solvents used as mobile phase.

Semi-quantitative analysis

Semi-quantitative data elaboration has highlighted two main ongoing ageing processes (Fig. 4): auto-oxidation of gallic acid to

Table 2
List of new degradation markers identified in the aged reference mock-ups. The most intense product ions are highlighted in bold. Retention time are referred to InfinityLab Poroshell 120 EC-C18 guard-column.

Ageing marker	t_R (min)	$[M-H]^-$	MS^2	Raw formula	Hypothesized structure
d_1	4.2	153.019	**108.020**	$C_7H_6O_4$	
d_2	8.9	247.025	**247.024**, 219.029, 191.034, 173.023, **145.031**, 119.049	$C_{12}H_8O_6$	
d_3	10.2	247.025	**247.024**, 219.029, **191.034**, 173.023, 145.031, 119.049	$C_{12}H_8O_6$	
d_4	11.5	259.025	**259.025**, 242.021, 213.019, 187.039, 159.045, 131.049	$C_{13}H_8O_6$	

ellagic acid, already described in our previous paper on a simplified model [10], and hydrolysis of poly-galloyl glucose species [7]. Briefly, the hydrolysis mechanism promotes a decrease in the relative percentage of bigger poly-galloyl glucose species (e.g., hepta-galloyl glucose, hexa- galloyl glucose, and penta- galloyl glucose), resulting in an increase of smaller glucose ones (e.g., tetra- galloyl glucose, tri- galloyl glucose, di- galloyl glucose, and mono-galloyl glucose) and gallic acid. Conversely, the autoxidation of gallic acid to ellagic acid results in the decrease of the former, promoting the increase of the latter. Considering poly-galloyl gallate species, the observed trends are more difficult to rationalise, since they can derive both from ageing pathways proceeding through condensation

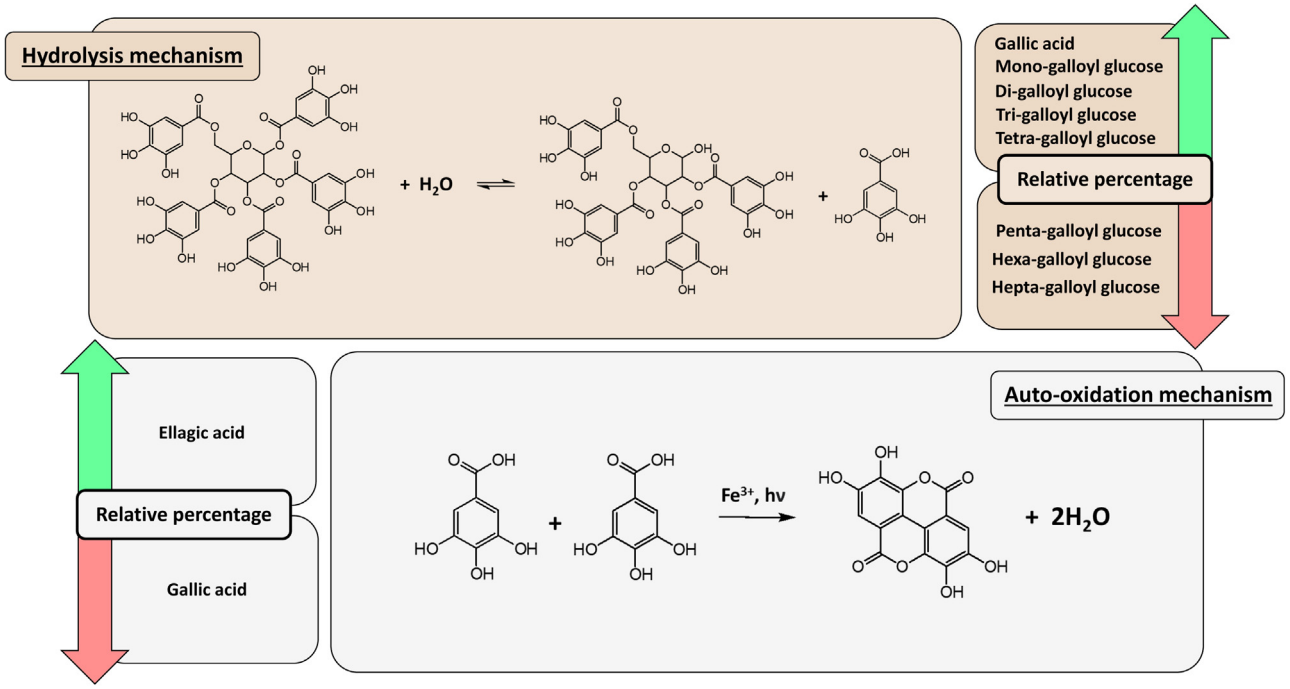


Fig. 4. Sketch of the ageing mechanisms observed by analysing the molecular profiles of mock-ups of iron gall inks.

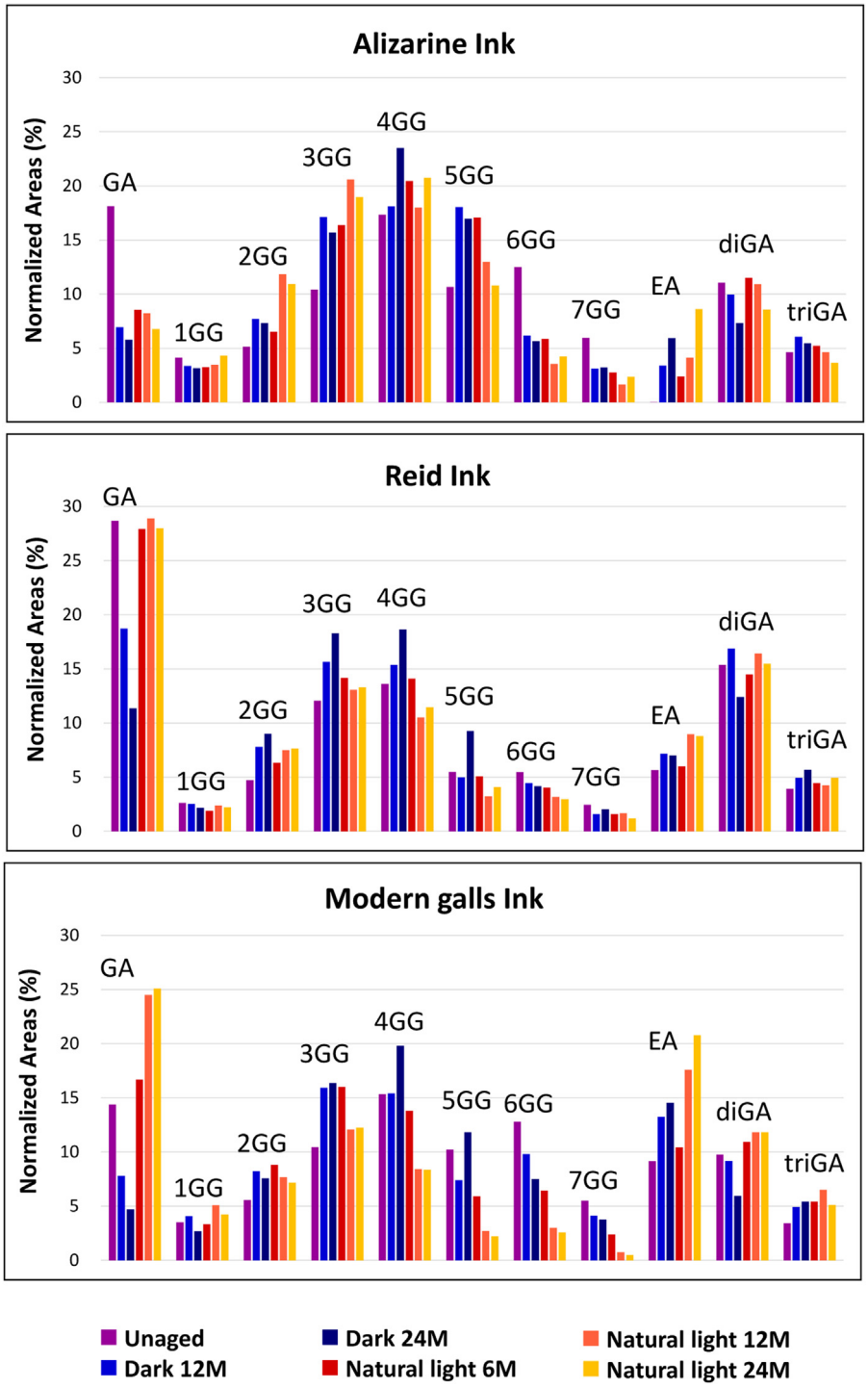


Fig. 5. Histograms of percentage peak areas of gallic acid (GA), mono-galloyl glucoses (1GG), di-galloyl glucoses (2GG), tri-galloyl glucoses (3GG), tetra-galloyl glucoses (4GG), penta-galloyl glucoses (5GG), hexa-galloyl glucoses (6GG), hepta-galloyl glucoses (7GG), ellagic acid (EA), digallic acid (diGA), and trigallic acid (triGA) in aged reference ink mock-ups.

of gallic acid [10] and from the hydrolysis of bigger oligomers that are not soluble in the extraction solution or detectable by HPLC-ESI-Q-ToF.

The degradation pathway observed for mock-ups stored in the dark (Fig. 5) is independent on the recipe, and the trends of the relative amounts of gallic, ellagic acids and poly-galloyl glucose species suggest that auto-oxidation mechanism is predominant for iron gall ink aged in the dark. Indeed, although the occurrence of hydrolysis mechanism can be proved by the decrease in the relative percentage of hexa- and hepta-galloyl glucoses (6GG and 7GG, respectively), the main pathway observed corresponds to the decrease of gallic acid induced by auto-oxidation. Thus, in these conditions the hydrolysis of poly-galloyl glucose species is probably kinetically unfavourable.

Conversely, the ageing performed by exposure to natural light (Fig. 5) exhibits recipe-dependent trends. In detail:

- Auto-oxidation prevails during the ageing of *alizarine ink*. Metallic iron and acetic acid added in the preparation could play a controversial role, the first by preventing auto-oxidation

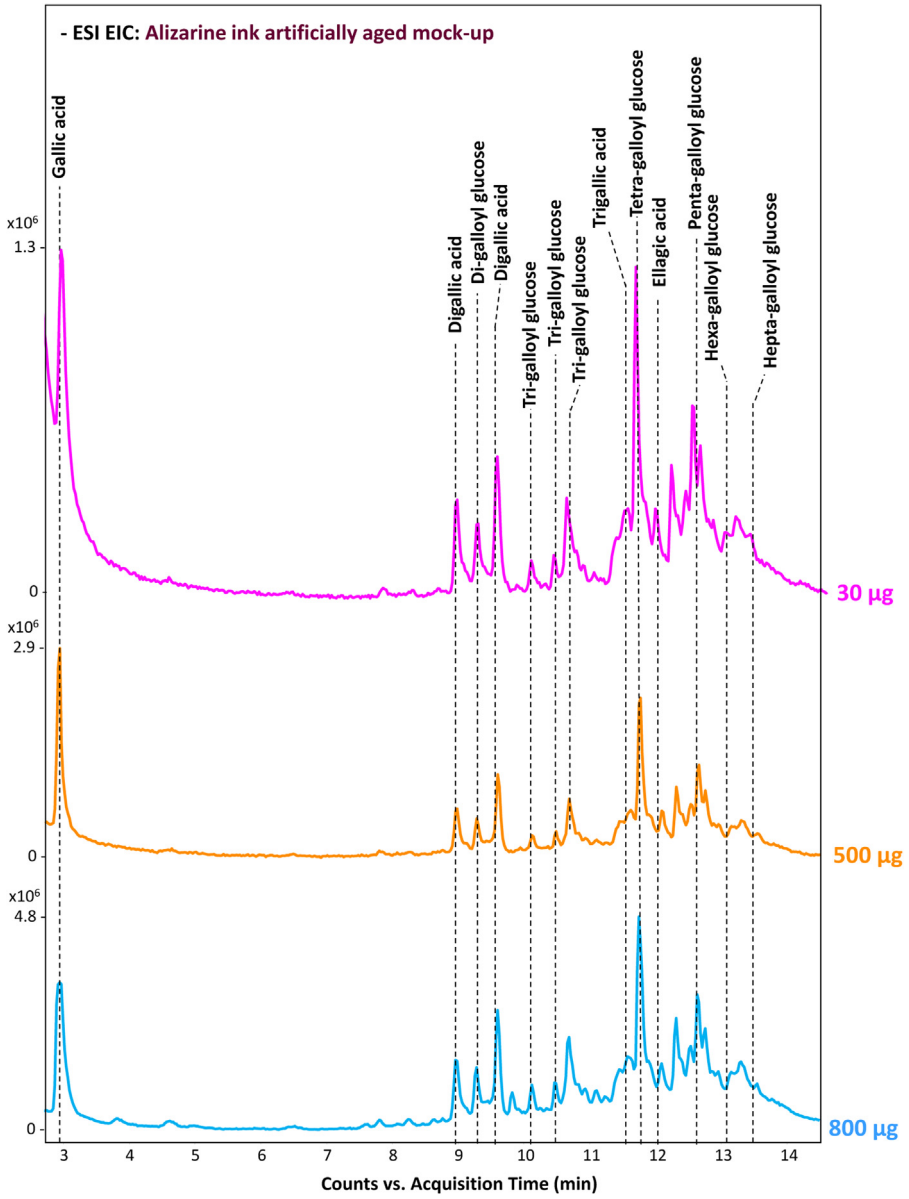


Fig. 6. Total Ion Chromatograms (TIC) acquired for EDTA-DMF extracts of alizarine artificially aged mock-up. Negative ionisation mode. 30 µg of reference mock-up (fuchsia profile), 500 µg of reference mock-up (green profile), and 800 µg of reference mock-up (light blue profile).

process and the latter promoting an initial hydrolysis of poly-galloyl glucoses. During ageing, auto-oxidation mechanism becomes prevalent. Indeed, the main trend detected is related to the decrease of gallic acid relative percentage, and this suggests a similar kinetic pathway to those described for dark ageing conditions.

- Hydrolysis of poly-galloyl glucose species is the main ageing mechanism during light exposure of *modern galls* ink mock-ups, promoting a decrease in 4GG, 5GG, 6GG, and 7GG and an increase in 3GG, 2GG, 1GG, and gallic acid relative amounts. The recipe in this case requires a seven-day period of pre-exposure of the decoction of oak galls to air, which could initially promote auto-oxidation. Once the ink is applied to the paper, hydrolysis mechanisms seem prevalent during the first weeks, while auto-oxidation restarts only after 12 months of light exposure, as proved by the notable increase in ellagic acid relative percentage. After one year, the relative amount of gallic acid reaches a plateau, suggesting that both mechanisms occur at the same rate (gallic acid is the product of the hydrolysis mechanism and the reagent in the auto-oxidation mechanism, as shown in Fig. 4).
- For *Reid ink* mock-ups, the relative percentage of gallic acid did not correlate with ageing time. Therefore, the free gallic acid produced by the hydrolysis of bigger poly-galloyl glucose species (4GG, 5GG, 6GG, and 7GG) was depleted by the auto-oxidation process yielding ellagic acid, preventing any significant change in the relative percentage of gallic acid from being detected. Thus, both mechanisms take place at a similar rate during ageing, proving that a one-day period of air exposure is not sufficient to promote either mechanism.

Tentative estimation of the minimum sample amount needed for analysis

The Total Ion Chromatograms (TIC) obtained for different quantities of aged alizarine ink mock-up (Section 2.6) are reported in Fig. 6. The aged molecular profile of alizarine ink, characterised by gallic acid, ellagic acid, poly-galloyl glucose, poly-galloyl gallate,

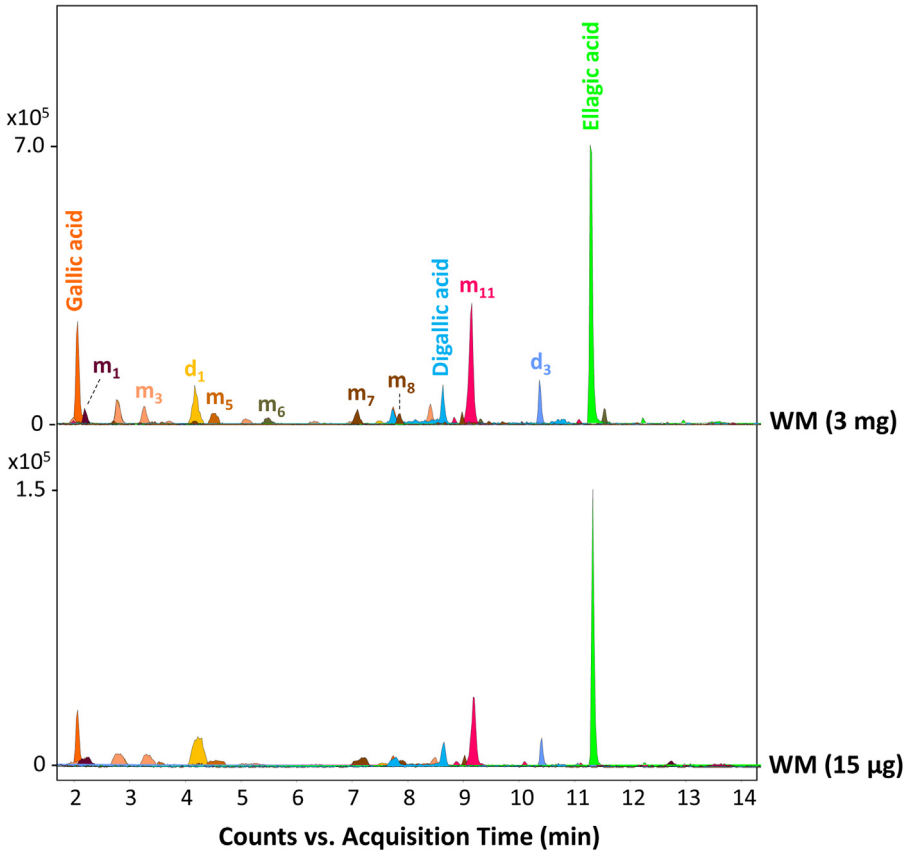


Fig. 7. HPLC-ESI-Q-ToF Extracted Ion Chromatogram (EICs) of gallic acid ($C_7H_6O_5$), digallic acid ($C_{14}H_{10}O_9$), ellagic acid ($C_{14}H_6O_8$), m_1 ($C_8H_6O_7$), m_3 ($C_8H_6O_6$), d_1 ($C_7H_6O_4$), m_5 ($C_8H_6O_5$), m_6 ($C_9H_8O_6$), m_7 ($C_{10}H_6O_6$), m_8 ($C_{10}H_6O_6$), m_{11} ($C_{10}H_8O_7$) and d_3 ($C_{12}H_8O_6$) obtained for EDTA-DMF extracts of Warsaw manuscript (WM, 3 mg and 15 µg).

and ageing markers reported in [10] and in this work, was successfully determined in all the chromatograms acquired, even for a sample weighting 30 µg treated with 150 µL of extraction solution. This estimation was further tested on a case study, as follows.

Case study

The HPLC-ESI-Q-ToF analyses carried out on the Warsaw manuscript samples (Fig. 7) enabled us to identify the typical molecular markers of iron gall inks. In detail, gallic acid, ellagic acid, digallic acids, and several ageing markers (e.g., m_1, m_3, d_1, m_8, m_{11}, d_3) were detected in both the sample amounts tested. The analysis performed on 3 mg of sample allowed us to determine also m_6 and m_7. Moreover, the dataset of molecular markers identified in the smaller sample (15 µg) successfully highlighted the presence of an iron gall ink, demonstrating the sensitivity of our methodology. The results confirmed the rough estimation made on the minimum amount of sample needed for a successful analysis, proving that it is possible to use micro-destructive techniques, such as liquid chromatography-tandem mass spectrometry, for the analysis of manuscripts and ink drawings on extremely reduced samples.

Conclusions

Three iron gall ink formulations were investigated to outline the link between ageing mechanisms and ink recipes. Additional degradation markers (with raw formula $C_7H_6O_4$, $C_{12}H_8O_6$, and $C_{13}H_8O_6$) to those observed for a model ink composed by gallic acid only were determined. The identification of these species in a manuscript or a drawing can be used as a proxy that iron gall ink was prepared from a decoction of galls, providing important information for authentication and dating purposes. Moreover, our study demonstrated that the relative percentage of gallic acid, ellagic acid, and poly-galloyl glucose provides important insights on the link between the historical recipe and the ageing of the polyphenolic profile. The first evidence is related to the ageing conditions. Indoor ageing in the dark promotes auto-oxidation mechanisms in all the formulations investigated and is thus recipe-independent. Differently, exposure to natural indoor light results in different ageing pathways impacted by the ingredients and procedure used in the ink production. This results in a promotion of auto-oxidation mechanism for inks containing additives capable of delaying oxidation process during the preparation (e.g., *alizarine ink*). Furthermore, an enhancement of the hydrolysis mechanism was observed for iron gall formulations that undergo seven days of exposure to air prior to filtration (e.g., *modern galls* ink). Instead, if only one day of exposure to air is used, both ageing mechanisms (auto-oxidation of gallic acid and hydrolysis of poly-galloyl glucose species) occur at a similar rate under exposure to natural indoor light (e.g., *Reid ink*). Finally, we demonstrated how liquid chromatography- tandem mass spectrometry analysis is a viable option for the analysis of historical manuscripts and drawings on micro sample, by detecting gallic and ellagic acids above LOD values in a 15 µg sample collected from an historical manuscript. In the same sample, ageing markers were also detecting, providing confirmation of the suitability of our studies performed on reference mock-ups.

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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.2024.02.012.

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