# Shedding light on the composition and degradation mechanism of dyes in historical ink's collection (19th-20th century)

Adele Ferretti[1], Ilaria Degano*[1,2], Stefano Legnaioli[3], Beatrice Campanella[3], Aurora Sainati[1], and Maria Perla Colombini[1]

[1] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via G. Moruzzi 13, 56124, Pisa, (Italy)

[2] Center for Instrument Sharing of the University of Pisa (CISUP), University of Pisa, Italy

[3] Consiglio Nazionale delle Ricerche (CNR), Istituto di Chimica dei Composti Organometallici, SS di Pisa, Via Moruzzi 1, 56124 Pisa, Italy

* ilaria.degano@unipi.it

## Abstract

Man has always used writing to be able to communicate, express, and disseminate his thoughts. In time, many different coloured extracts of plant and animal origin have been used to produce inks; after the development of synthetic chemistry, artificial and synthetic dyes started to be widely exploited. The end of the 19th century marked great technological and industrial innovations in commercial production of artists' materials. To reveal ink formulations and build a database of red inks by different producers, we developed a multi-analytical approach and investigated a collection of writing inks produced in France in the late 19th – early 20th century. The materials used as binders, additives, dyes, and pigments have been investigated by high performance liquid chromatography coupled with diode array and tandem mass spectrometric detectors (HPLC-DAD-$MS^2$), in situ derivatisation pyrolysis coupled with gas chromatography-mass spectrometry (Py(HMDS)-GC/MS), surface enhanced Raman (SERS, TLC-SERS) and X-Ray Fluorescence spectroscopies. Several dyes and pigments were detected, showing that the French ink's formulations of the early days of synthetic dye industry were based on *rhodamine B* and *6G*, *eosin Y*, *rose Bengal* and *methyl* or *crystal violet*. Instead, as binder and additives only *gum Arabic* and *shellac resin* have been identified, respectively. Mass spectrometry also allowed us to detect possible by-products of the synthesis of ink's dyes and even early degradation products, that can be used for ink identification in historical writings and drawings. Our studies can pave the way to investigate inks in historical samples by introducing ultra-sensitive chromatographic and mass spectrometric methods in the array of analytical tools available to the chemist.

**Keywords:** Historical red inks; Raman spectroscopy; TLC-SERS; HPLC-ESI-Q-ToF; synthetic organic pigments

## 1. Introduction

The investigation of historical and archaeological artefacts, such as pottery, writing instruments, inks, and textiles is influential in improving society's understanding of the past, present and future [1,2]. The technique and the art of writing and drawing has progressed over the centuries, along with the supports and materials necessary for its expression [3]. Although the composition of inks may differ based on the desired colour and specific products, they are all composed of three main components [4,5]: the *liquid vehicle*, or the ink's portion which suspends and transports the dye to the paper support; *colourants*, that impart a well-defined colour to the writing material; *additives,* or rather substances that are specifically added to enhance writing performances. The early development of inks involved the use of natural or artificial pigments [6–8], such as *lampblack* [9], used to prepare carbon-based inks, or organic pigments like *tannins*, *hematein* and *sepia*, employed in iron gall [10,11], logwood [12] and sepia ink [13] formulations, respectively. The 19$^{th}$ century was a period of great technological and industrial innovations in this field. The increase in the global literacy rate caused a dramatic change in the ink manufacture, leading to industrial production. Furthermore, in 1856 Perkin discovered Mauveine [14,15], marking the beginning of the synthetic organic pigment (SOPs) industry. In this context, ink formulations began to change. Thus, if in the 19$^{th}$ century both natural and synthetic pigments played a fundamental role in the production of inks, starting from the early 20$^{th}$ century the use of SOPs became a trend in art and in ink formulations [16]. SOPs allowed a wide range of different colours to be obtained in a simpler and more cost effective way, and writing materials to perform better than inks prepared from natural dyes, opening up a new and fascinating landscape for ink's manufactures. Among the most important classes of SOPs introduced on the market in the late 19$^{th}$ century, *triarylmethines*, *xanthenes, thiazine* and *hydrazone dyes* (Figure 1) were widespread in ink's recipes. Triarylmethines dyes are nowadays known as the most widely used early synthetic dyes. All the SOPs belonging to this class are characterized by bright colours, given by three conjugated aromatic rings linked to a central atom of carbon and various auxochromic substituents [17]. Thanks to their versatility, triarylmethines dyes have been widely used up to now in textiles, artworks, ballpoint pens and writing inks [18–20]. Xanthenes dyes, obtained through an intramolecular cyclization of triarylmethines bridged with an oxygen atom [17], are fluorescent dyes that have been very attractive for their brilliant hues [21]. Consequently, xanthenes have been broadly exploited in the artistic field, for the printing industry, but also in the cosmetic, pharmaceutical and food fields [18,22,23]. However, both of these classes exhibit a strong tendency to fade upon light exposure, and the photostability of some representative xanthene dyes has been discussed in the literature [18,24,25]. Hydrazone pigments, characterised by 1-arylhydrazone-2-naphthol system [26], constitute the largest group of synthetic organic pigments ever produced [27]. On account of their brilliant shades, excellent lightfastness and high tinting power, these compounds have been employed for several applications, such as leather, plastic, medicine, food and ink's industry [27–29]. Thiazine dyes are tricyclic and heterocyclic compounds with one atom of sulphur and one nitrogen in the central ring [30]. The high luminescence and

photoconductivity of these SOPs have promoted their spreading as dyes in paper, writing inks, lake-pigments, textiles, or in more recent applications for microscope cell staining, pharmaceuticals, and photo galvanic cells [30–33].

From an analytical point of view, the identification of inks in drawing or manuscript samples is challenging, to a large extent due to the numerous recipes used over the centuries. Furthermore, the ageing and fading processes promote a variation in the chemical profile of the inks, providing a different molecular composition over time compared to the original one. This phenomenon, reported in the literature for crystal violet-based inks [24] and for a model iron gall ink [34], is particularly evident when SOPs, amongst the most fragile materials in works of art and documents, are the mainly dye component of the ink's formulations. Hence, the analysis of historical artistic materials is of utmost importance to improve our understanding of manufacturing techniques, as already showed in the literature for the study of tube paints, felt-tip pens, crayons, historical pigments and ink's collection [16,18,35]. At the same time, the study of historical collections could allow to detect dyes/pigments degradation products, enabling heritage science to gain information on synthetic pathways and degradation process, and help planning sustainable conservation strategies. Several techniques have been exploited for the detection of synthetics organic pigments in the literature, such as Raman or surface enhanced Raman spectroscopy (SERS) [36–38], thin layer chromatography (TLC) coupled to SERS analysis [39,40], pyrolysis coupled to gas chromatography – mass spectrometry [18,20] and capillary electrophoresis [41]. In the last decade a general consensus identified high performance liquid chromatography (HPLC) coupled to several detectors as the technique par excellence for the analysis of SOPs and their by-products [15,18,42–44] since its high sensitivity and selectivity allows both the determination of trace compounds and of mixture of dyes.

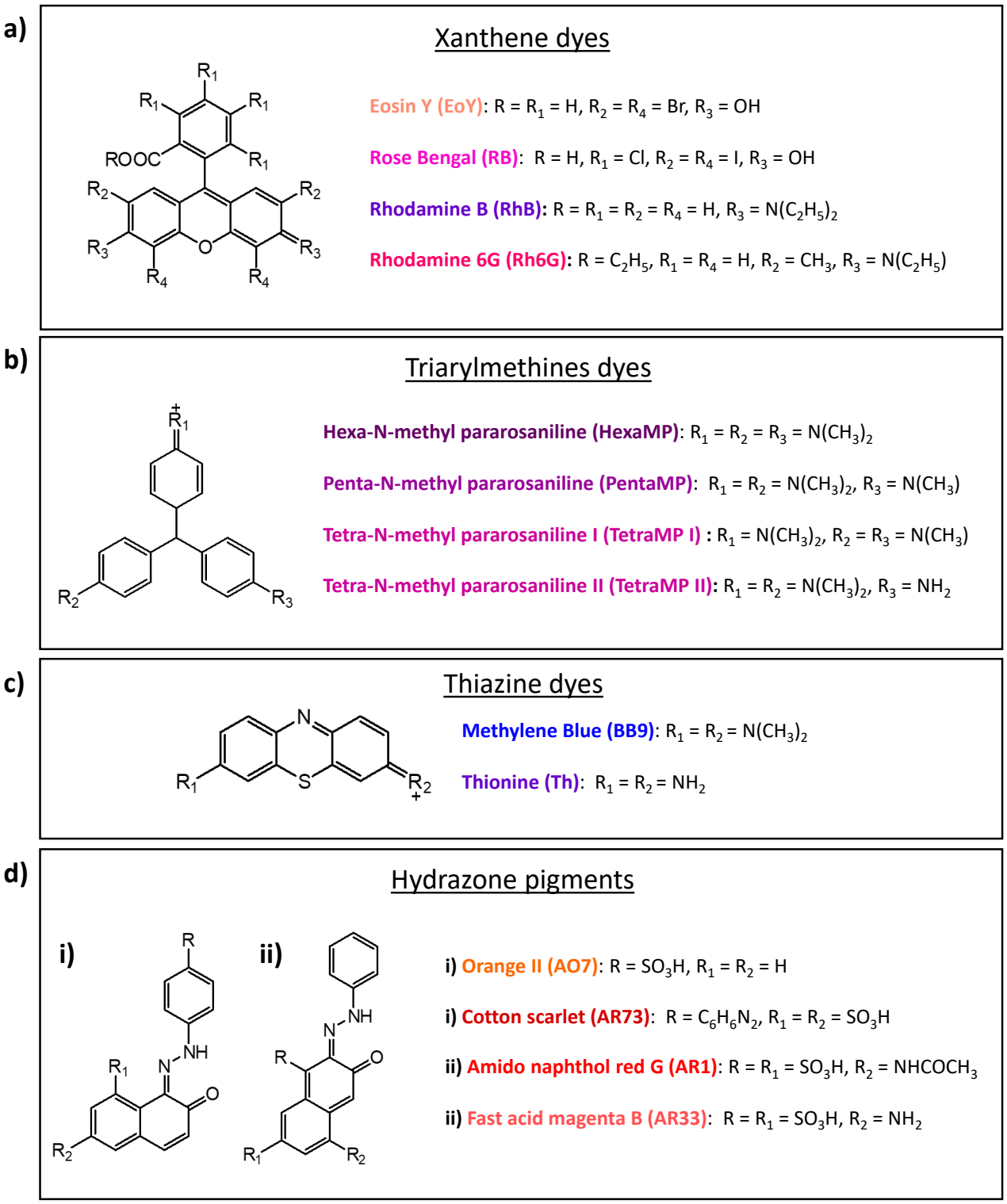


Figure 1: Chemical structures of **a)** xanthene dyes; **b)** triarylmethines dyes; **c)** thiazine dyes; **d)** hydrazone pigments.

## 2. Research Aim

This work aims to reveal trends in historical ink formulations, to develop a multi-analytical approach able of fully characterising the ink composition, and to highlight degradation markers enabling the identification of

the colourants in aged and possibly faded historical ink samples. Thus, a multi-analytical approach was developed for the analysis of historical inks and applied to a collection of writing materials produced in France between the late 19$^{th}$ and the early 20$^{th}$ century. The array of analytical techniques used comprises surface enhanced Raman scattering spectroscopy (SERS), thin layer chromatography coupled to SERS (TLC-SERS), X-Ray fluorescence spectroscopy (XRF), high performance liquid chromatography coupled to diode array detection and tandem mass spectrometry (HPLC-DAD, HPLC-ESI-Q-ToF), and in situ derivatisation pyrolysis coupled to gas chromatography-MS (Py(HMDS)-GC/MS). Furthermore, HPLC-ESI-Q-ToF analysis also allowed us to perform studies on the degradation products of ink dyes. The collected results provided valuable information for art technological studies, dating and authentication processes.

## 3. Materials and Methods

### 3.1 19$^{th}$-20$^{th}$ century inks

In the present work an historical red ink's collection from the late 19$^{th}$ and the early 20$^{th}$ century was analysed. The collection is composed by sixteen inks produced in France from different ink's manufacturers, and they were collected from an antique dealer (Vintage Waterman Pens, USA). The inks were contained in their original glass bottles, and their chromatic shades ranges from pale pink to bright purple and from peach orange to scarlet red. The inks are today in the form of a powder. To assess whether our procedure would be able to detect the dye/pigment components in an historical sample, the powders were collected, and several sample pre-treatments were applied. The inks analysed were classified and the samples, with the associated samples code, label, photos, colours, and results of the analyses are listed in Table 1.

Table 1: Historical ink's collection from 19$^{th}$-20$^{th}$ century. The following information are reported for each ink: code of the sample used during the analysis (samples code), name of the ink and/or manufacturer reported on the bottle labels (label), photo of the ink bottles (photo), and colours of the ink mock-ups obtained in accordance with section 3.3 (colour).

| Samples code | Label | Photo | Colour |
|---|---|---|---|
| **1** | Encre Écarlate; New Red Ink; Spécialement préparée pour les plumes métalliques; N. Antoine & Fils; Paris | | |
| **2** | Encre Rouge Écarlate; Edouard Devillers; Paris | | |

| | | | |
|---|---|---|---|
| **3** | Encre a tampon; sans huile; rouge; cette encre sèche rapidement et donne de très belles empreintes; Bagnolet Paris. | | Ink's 3 bottle was found empty. |
| **4** | Vermillon; Indélébile; Yang Tse; J.M. Paillard; Paris | | |
| **5** | Olympic; Encre De Chine | | |
| **6** | Bayard; Encre Supérieure | | |
| **7** | Manufacture Parisienne; Au Chat Noir; Encre Rouge; Paris | | |
| **8** | Vermillon; Indélébile; Yang Tse; J.M. Paillard; Paris | | Ink 8 was a replica of ink 4. |
| **9** | Encre a Tampon; Tiflex; Supérieure; Sans Huile | | |
| **10** | Encre Écarlate; New Red Ink; Spécialement préparée pour les plumes métalliques; Encre Antoine; Paris | | |

| | | | |
|---|---|---|---|
| **11** | Encre Rouge Supérieure | | |
| **12** | Encre De Couleur; Parfumée; Carminée; Médailles D'Or; Paris 1889 & 1900; Adrien Maurin Paris | | |
| **13** | Papeterie Generale Des Ecoles; Encre Couleur Superieure; Boudignon Sartiaux; Paris | | |
| **14** | Encre Gauloise; Carminée Supérieure; V. Chantraine Paris | | |
| **15** | Encre Modèle Rouge; R. Barth | | |
| **16** | Pourpre; Couleurs Liquides Indélébiles; Tien-Tsin; Encres Antoine | | |

### 3.2 Raman, Surface Enhanced Raman Scattering (SERS) and Thin Layer Chromatography-Surface Enhanced Raman Scattering (TLC-SERS)

The synthesis of the silver nanoparticles (AgNPs) was performed according to Lee & Meisel procedure [45]. SERS measurements were made on a Renishaw InVia instrument coupled with an optical Leica DLML microscope, equipped with a NPLAN objective 20× and 50×. Prior to SERS analysis, 1-2 mg of ink's powder were extracted in an ultrasonic bath (60 °C, 15 minutes) with 50 µL of ethanol. SERS measurements were made by mixing at room temperature on a glass slide 5 µL of AgNPs, 1 µL of $KNO_3$ (aggregating agent) and 1 µL of sample extract in ethanol. Spectra were acquired immediately by focusing on the top of the drop.

For TLC-SERS, the separation took place on Silica gel 60 plates (Supelco) as stationary phase and a isopropyl alcohol:acetone:ammonia (11:1:2) as eluent. 1-2 mg of ink's powder was suspended in 50 µL of acetone∼2 µL of each extract was deposited onto the TLC plate by means of a glass capillary and eluted in a glass-developing chamber. The separated spots were visualized under a UV lamp at 254 and 365 nm. The separated components were analysed directly on the TLC plate by placing 1 µL of AgNPs on top of each spot [39].

More details can be found in [39] and in Supplementary Information S1.1-4.

### 3.3 X-Ray Fluorescence (XRF)

XRF experiments were performed using the Elio portable XRF Analyzer (XGLab, Italy), equipped with a 10-40 keV/200 µA X-ray tube (Rh electrode, 1 mm collimated beam on the sample) and a large-area Energy Dispersive Si-Drift detector (130 eV FWHM at Mn Kα). All the XRF spectra were acquired for 90 s with 40 kV of voltage and 80 µA of current. Prior to XRF measurements, the ink's powder (1-2 mg) was dispersed in methanol. XRF analysis were performed on ink's mock-ups prepared by spreading the ink's dispersion on Whatman filter paper (USA, grade 42, diameter 110 mm, pure cellulose).

### 3.4 High Performance Liquid Chromatography (HPLC-DAD-MS$^2$)

The HPLC-DAD system consists of a PU-2089 quaternary pump equipped with a degasser, an AS-950 autosampler and an MD-2010 spectrophotometric diode array detector (DAD) (all modules Jasco International Co., Japan). The HPLC-ESI-Q-ToF consists of an 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) was used. Prior to HPLC analysis, 100-200 µg of ink's powder were suspended in 300-400 µL of 0.1% EDTA aqueous solution/DMF (EDTA-DMF, 1:1, v/v), and sonicated for 1 h at 60 °C. The supernatant was purified with PTFE syringe filters (4 mm thickness and 0.45 µm pore diameter, Agilent), and then injected in the chromatographic system.

Further details can be found in [46] or in the Supplementary Information file S1.5.

### 3.5 Pyrolysis coupled to gas chromatography-mass spectrometry (Py-GC/MS)

Py-GC/MS analysis was performed using a multi-shot pyrolyzer EGA/PY-3030D (Frontier Lab, Japan) coupled to a 8890 gas chromatograph, combined with a 5977B mass selective single quadrupole mass spectrometer detector (Agilent Technologies, USA). To characterize the ink's binder, pyrolysis with in-situ silylation using 4 µL of hexamethyldisilazane (HMDS) as derivatizing agents was carried out. Further details can be found in [47] and in Supplementary Information file S1.6.

## 4. Results and discussion

Our studies lead to a broad overview of trends in ink's composition in $19^{th}$ – $20^{th}$ century in France. The results are summarized in Table 2 and will be presented in detail in the following paragraphs. The identification of both the dyes/pigments and binders was performed by comparison with standard and reference materials or based on matching data found in the literature. Instead, degradation products related to dye components, and never described before in the literature, were characterized through MS and $MS^2$ spectra interpretation. All the molecular markers determined by SERS, TLC-SERS, HPLC-DAD-$MS^2$ and Py(HMDS)-GC/MS are summarized in Tables S.3-6, respectively (Supplementary Information file).

SERS spectroscopy was first carried out to obtain a preliminary screening of the ink's dyes and allowed us to split the historical inks into three groups (as presented in Table 2): the first, encompassing the inks containing triarylmethine pigments (methyl or crystal violet); the second and third, comprising the inks based on xanthene pigments with halogen substituents, namely rose Bengal and eosin Y; the fourth one, containing the amino-substituted xanthene pigment rhodamine. The specific wavenumbers obtained for each standard pigment and French ink are reported in Tables S.2-4 (Supplementary Information file). In detail,

- French Inks 1, 6, 9 and 14 were preliminary classified as **methyl** or **crystal violet-based inks** (Figure 2a). Methyl (Mv) and crystal violet (Cv) belong to the class of *triarylmethine pigments*: Mv (C.I. 42535), introduced on the market in 1861, consists of a mixture of tetra-, penta- and hexa-*N*-methyl pararosaniline, while Cv (C.I. 42555), commercialized in 1883 by Kern and Caro, contains hexa-N-methyl pararosaniline only [48]. For their differentiation, a separative technique is necessary;
- French Ink 12 was identified as **rose Bengal-based ink** (Figure 2b). Rose Bengal (Acid Red 94, C.I. 45440), first synthesised by Gnehm in 1882, belongs to the class of xanthenes, imparts a pink colour and is a halogen-containing dye-pigment (chlorine and iodine atoms) [49];
- French Inks 2, 7, 11, 13 and 15 were characterized as **eosin Y-based inks** (Figure 2c). Eosin Y (Acid Red 87, C.I. 45380) is an acid dye produced since 1873 thanks to Heinrich Caro. It is a brominated fluorescein which is normally orange in its insoluble acid form [50,51];
- French Inks 4, 5, 10 and 16 were determined as **rhodamine-based inks** (Figure 2d). Specifically, rhodamine B (Basic Violet 10, C.I. 45170) was detected in ink's formulations 4, 5, and 16, while rhodamine 6G (Basic Red 1, C.I. 45160) was determined in French Ink 4 and 10. Both dyes are basic xanthenes: rhodamine 6G is characterized by a scarlet tone and was patented in 1887, while rhodamine B is a highly fluorescent violet dye commercialized in 1887 by Cérésole [52,53].

To obtain additional information regarding the trend's composition of 19$^{th}$ - 20$^{th}$ century ink's formulations, further analyses were carried out with liquid chromatography techniques (TLC-SERS, HPLC-DAD, HPLC-ESI-Q-ToF), since a separation step is fundamental for the analysis of complex mixtures such as writing inks.

Table 2: Summary of the results of the analyses of French inks (if the results did not significantly differ from the analytical blank, the corresponding cell is marked with an x).

| | Ink | SERS | TLC-SERS | HPLC-DAD | HPLC-ESI-Q-ToF | | Py-GC/MS | Summary | |
|---|---|---|---|---|---|---|---|---|---|
| Methyl or crystal violet- based inks | 1 | Mv or Cv | AR73 | AR73 | (+) | Cv | x | Dyes | Cotton scarlet (AR73), crystal violet (Cv) |
| | | | | | (-) | AR73 | | Binder, Additives | x |
| | 6 | Mv or Cv | x | AR33, AR1 | (+) | Mv or degraded Cv, BB9, BB9-Me | Arabic gum (?), AR1 | Dyes | Amido naphthol red G (AR1), fast acid magenta B (AR33), methylene blue (BB9), methylene blue photo-oxidation products (BB9-Me), degraded crystal violet (Cv) or methyl violet (Mv) |
| | | | | | (-) | AR33, AR1 | | Binder, Additives | Arabic gum? |
| | 9 | Mv or Cv | AR73 | AR73 | (+) | Cv | AR73 | Dyes | Cotton scarlet (AR73), crystal violet (Cv) |
| | | | | | (-) | AR73 | | Binder, Additives | x |
| | 14 | Mv or Cv | AR73 | AR73 | (+) | Cv | x | Dyes | Cotton scarlet (AR73), crystal violet (Cv) |
| | | | | | (-) | AR73 | | Binder, Additives | x |
| Eosin Y or rose Bengal- based inks | 2 | EoY | EoY | EoY, DBEo | (+) | x | x | Dyes | Eosin Y, eosin Y by-products (DBEo, pseudo EoY) |
| | | | | | (-) | EoY, DBEo, pseudo EoY | | Binder, Additives | x |
| | 7 | EoY | EoY | EoY, DBEo | (+) | x | x | Dyes | Eosin Y, eosin Y by-products (DBEo, pseudo EoY) |
| | | | | | (-) | EoY, DBEo, pseudo EoY | | Binder, Additives | x |
| | 11 | EoY | EoY | EoY, DBEo | (+) | x | EoY | Dyes | Eosin Y, eosin Y by-products (DBEo, pseudo EoY) |
| | | | | | (-) | EoY, DBEo, pseudo EoY | | Binder, Additives | x |
| | 12 | RB | RB | EoY, DBEo | (+) | x | x | Dyes | Eosin Y (EoY), eosin Y by-products (DBEo, 2DBEo, pseudo EoY), rose Bengal (RB), rose Bengal by-products or degradation markers (DCRB, 2DCRB) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | (-) | EoY, DBEo, 2DBEo, pseudo EoY, RB, DCRB, 2DCRB | | Binder, Additives | x |
| | 13 | EoY | EoY, RB | EoY, DBEo, AR33, AR1 | (+) | x | EoY | Dyes | Eosin Y (EoY), eosin Y by-products (DBEo, 2DBEo, pseudo EoY), rose Bengal (RB), rose Bengal by-product or degradation marker (DIRB), fast acid magenta B (AR33), amido naphthol red G (AR1) |
| | | | | | (-) | EoY, DBEo, 2DBEo, pseudo EoY, RB, DIRB, AR33, AR1 | | Binder, Additives | x |
| | 15 | EoY | EoY | EoY, DBEo | (+) | x | EoY | Dyes | Eosin Y (EoY ), eosin Y by-products (DBEo, 2DBEo, pseudo EoY) |
| | | | | | (-) | EoY, DBEo, 2DBEo, pseudo EoY | | Binder, Additives | x |
| Rhodamine- based inks | 4 | RhB, Rh6G | RhB, Rh6G | RhB, Rh6G, DERhB, bisDERhB | (+) | RhB, Rh6G, DERhB, DERh6G, bisDERhB, bisDERh6G, pseudo RhB | Shellac, RhB | Dyes | Rhodamine B (RhB), rhodamine 6G (Rh6G), rhodamine photo-oxidation products (DERhB, DERh6G, bisDERhB, bisDERh6G), rhodamine B by-product (pseudo RhB) |
| | | | | | (-) | Shellac | | Binder, Additives | Shellac resin |
| | 5 | RhB | RhB | RhB, AO7, DERhB, bisDERhB | (+) | RhB, DERhB, bisDERhB, pseudo RhB | Shellac, RhB, AO7 | Dyes | Rhodamine B (RhB), rhodamine B photo-oxidation products (DERhB, bisDERhB), rhodamine B by-product (pseudo RhB), orange II (AO7), orange 2R (AO8) |
| | | | | | (-) | Shellac, AO7, AO8 (?) | | Binder, Additives | Shellac resin |
| | 10 | Rh6G | RhB, Rh6G, EoY | RhB, Rh6G, EoY, AR1, AR33, DERhB, bisDERhB | (+) | RhB, Rh6G, DERhB, DERh6G, bisDERhB, bisDERh6G, trisDERhB, trisDERh6G, Rh, | x | Dyes | Rhodamine B (RhB), rhodamine 6G (Rh6G), rhodamine photo-oxidation products (DERhB, DERh6G, bisDERhB, bisDERh6G, trisDERhB, trisDERh6G, Rh), eosin Y (EoY), eosin Y by-products (DBEo, 2DBEo, pseudo EoY), fast acid magenta B (AR33), amido naphthol red G (AR1) |
| | | | | | (-) | EoY, AR1, AR33 | | Binder, Additives | x |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **16** | RhB | RhB | RhB, DERhB, bisDERhB | **(+)** | RhB, DERhB, bisDERhB, pseudo RhB | Shellac, RhB | **Dyes** | Rhodamine B (RhB), rhodamine B photo-oxidation products (DERhB, bisDERhB), rhodamine B by-product (pseudo RhB) |
| | | | | | **(-)** | Shellac | | **Binder, Additives** | Shellac resin |

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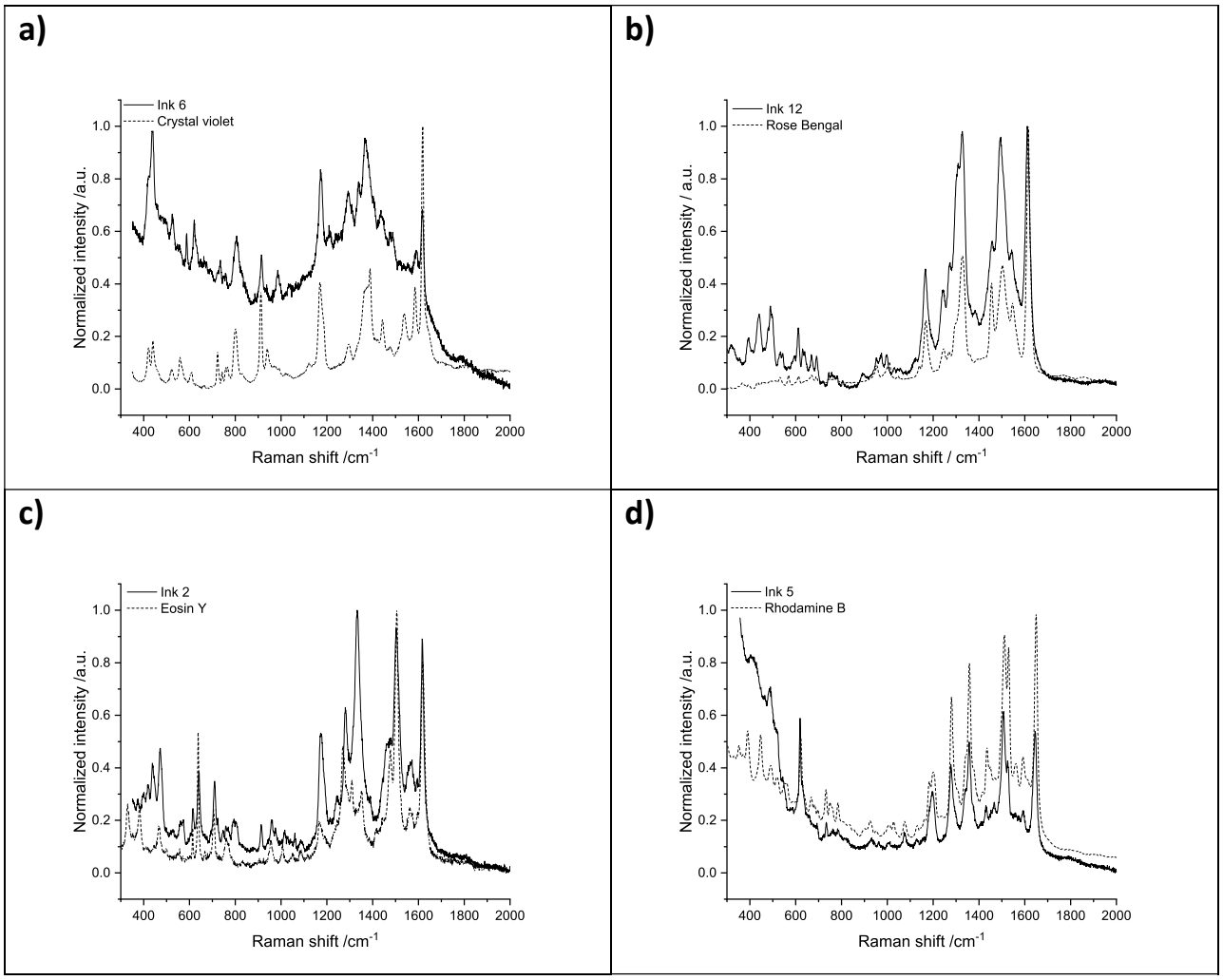


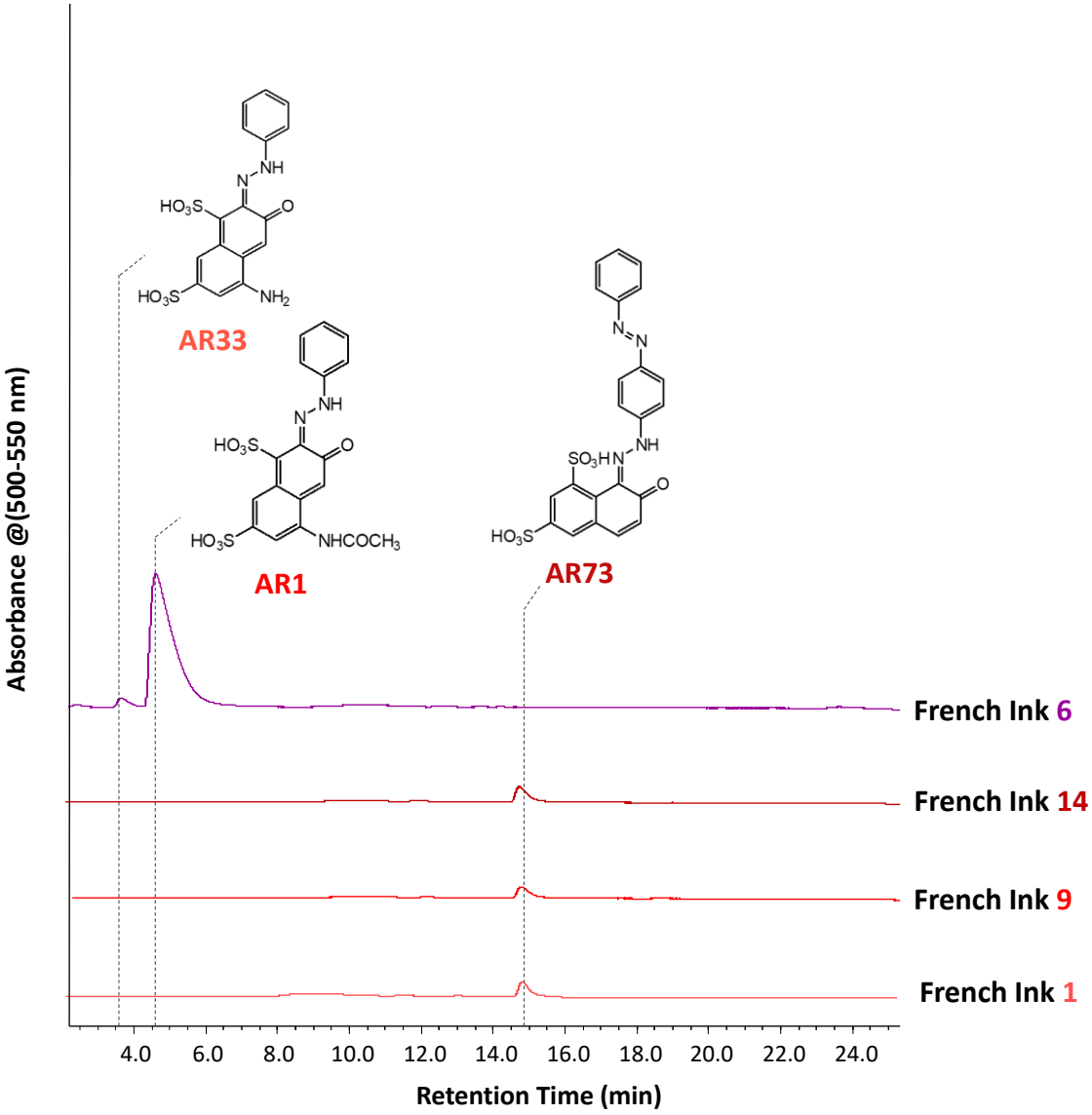

Figure 2: SERS spectra of a) French Ink 6 (crystal violet-based); b) French Ink 12 (rose Bengal-based); c) French Ink 2 (eosin Y-based); d) French Ink 5 (rhodamine-based). Reference spectra are reported as dotted lines.

### 4.1.1 Methyl/crystal violet-based inks

Concerning the inks determined as methyl violet (Mv) or crystal violet (Cv) based, further information was achieved by liquid chromatography techniques. TLC-SERS provided the identification of *cotton scarlet* (AR73) in French Inks 1, 9, and 14. Thus, the technique did not confirm SERS results, and this could be due to the non-optimal chromatographic conditions adopted for basic triarylmethine dyes. The presence of AR73 in French Inks 1, 9, and 14 was further confirmed by HPLC-DAD (Figure 3), which allowed us to detect also *fast acid magenta B* (AR33, 3.5 min) and *amido naphthol red G* (AR1, 4.8 min) in French Ink 6. These three acid dyes (AR73, AR1, AR33) were also identified by HPLC-ESI-Q-ToF (negative ionisation mode) results. According to the current knowledge, it could be possible that AR33 was identified as a synthetic intermediate of AR1 [63], since the two dyes only differ in the presence of an acetyl substituent. Alternatively, the formation of AR33 could be the result of the deacetylation of AR1, since ageing phenomenon involving a deacetylation step are known in the literature [64]. Further

information was achieved by liquid chromatography-tandem mass spectrometry in positive ionisation mode (Figure 4), which finally provided the identification of French Inks 1,9 and 14 as Cv-based formulations, confirming the results of SERS spectroscopy. Instead, in French Ink 6 the recognition of the demethylated forms of hexa-N-methyl pararosaniline (Hexa MP, 20.7 min; Penta MP, 19.5 min; Tetra MP, 18.2 and 17.7 min; Tri MP, 16.9 and 16.4 min) allowed us to hypothesize the use of Mv in the formulation, or that the originally present Cv underwent the photodegradation pattern described in the literature [18]. Moreover, a blue dye belonging to thiazine dye class, *methylene blue* (BB9, 11.8 min), was determined in French Ink 6. The high selectivity and sensitivity of liquid chromatography-tandem mass spectrometry also provided the identification of an unknown compound, which was characterized as the demethylated form of BB9 (BB9-Me, 11.1 min) through the interpretation of its tandem mass spectrum (Figure S.1). BB9-Me could be a synthetic by-product, or be due to a degradation process, as typical photo-oxidation pathways entailing the loss of methyl groups are known for dyes featuring substituted amino functionalities [18,30].

Finally, Py(HMDS)-GC/MS analysis allowed us to determine levoglucosan (in its derivatized form) in French Ink 6 (Figure S.2). Levoglucosan is the pyrolytic marker of several materials, and in the heritage field it could be ascribed to the presence of cellulose or polysaccharidic gums, such as gum Arabic, mesquite gum etc. Since gum Arabic was the most used binder in historical inks [6], and given the context of the analysis, the identification of levoglucosan suggests the presence of this binder in French Ink 6 formulation. In addition, pyrolytic markers related to amido naphthol red G and cotton scarlet were identified in French Ink 6 and 9 [51,65], respectively, while for French Ink 1 and 14 no additional information were obtained with respect to the analytical blank.

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230 Figure 3: HPLC-DAD chromatograms (500-550 nm) of French Ink 1 (light red profile), 9 (red profile), 14 (dark red profile),
231 and 6 (violet profile). All chromatograms are presented in the same scale and are stacked for purpose of clarity.

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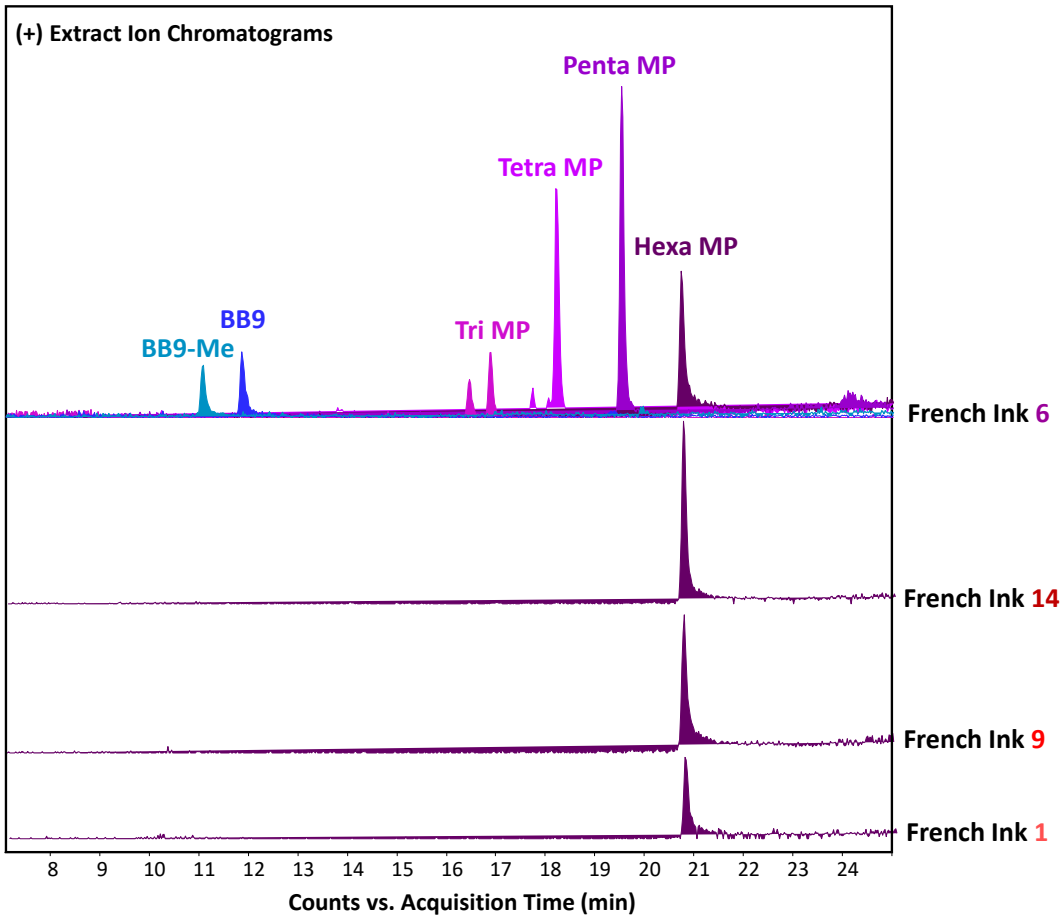


Figure 4: HPLC-ESI-Q-ToF Extract Ion Chromatograms (EIC) obtained by plotting the counts in function of time of the ions corresponding to the molecular ions of the single species detected in the EDTA-DMF extracts of the French Inks 1, 9, 14 and 6. The detected species, highlighted in the chromatograms, are hexa-N-methyl pararosaniline (Hexa MP, $C_{25}H_{30}N_3^+$), penta-N-methyl pararosaniline (Penta MP, $C_{24}H_{28}N_3^+$), tetra-N-methyl pararosaniline (Tetra MP, $C_{23}H_{26}N_3^+$), tri-N-methyl pararosaniline (Tri MP, $C_{22}H_{24}N_3^+$), methylene blue (BB9, $C_{16}H_{18}N_3S^+$) and demethylated methylene blue (BB9-Me, $C_{15}H_{16}N_3S^+$). Positive acquisition mode. All chromatograms are presented in the same scale and are stacked for purpose of clarity.

### 4.1.2 Eosin Y and Rose Bengal-based inks

From SERS analysis *eosin Y* (Eo Y) was determined in French Inks 2, 7, 11, 13 and 15, while *rose Bengal* (RB) was detected in French Ink 12 only. The results acquired with SERS spectroscopy were all confirmed by TLC-SERS, which also additionally provided the identification of rose Bengal in French Ink 13 (Figure 5). The presence of the tetra-brominated dye (Eo Y) has also been demonstrated by XRF spectroscopy (Figure S.3), which allow to identify bromine in all the Eo Y-based inks spectra (French Inks 2, 7, 11, 13 and 15). Instead, XRF analysis did not provide any information related to chlorine and iodine atoms, typical of rose Bengal dye. Further information were obtained by high performance liquid chromatography analysis: HPLC-DAD (Figure S.4) and HPLC-ESI-Q-ToF in negative ionisation mode (Figure 6) consistently detected eosin Y and its debrominated molecular markers [58] in all the Eo Y and RB- based inks, allowing us to further determine eosin Y in French Ink 12. Moreover, *amido naphthol red G* (AR1) and *fast acid magenta B* (AR33) were identified in French Ink 13 through HPLC techniques. Besides, HPLC-ESI-Q-ToF provided us with the identification of further degradation products or synthetic by-products. The examination of the exact masses, isotopic patterns, and tandem mass spectra of the precursor ions allowed us to hypothesize the molecular structure of several secondary components of the dye mixture. Specifically, chlorine and iodine containing species were assigned as Rose Bengal's synthesis by-products or degradation markers. The dechlorinated (DCRB, 25.0 min), bis-dechlorinated (2DCRB, 24.4 min) and deiodinated (DIRB, 24.7 min) dye molecules were all detected. Considering the literature studies on eosin Y [58], the hypothesis of subsequent dechlorination or deiodination from the Rose Bengal structure is a plausible degradation pathway. Furthermore, dechlorinated derivatives of rose Bengal were already detected by Tamburini et al. in a textile sample book dated 1893 [59]. Interestingly, in all the Eo Y-based inks (French ink 2, 7, 11, 12, 13 and 15) a further unknow species characterised by the isotopic pattern of a dye containing four bromine atoms was detected. The exact mass of the molecular ion ($[M-H]^-$ = 716.736) could be attributed to the molecular formula $C_{24}H_{14}Br_4O_6$, proving a relation with eosin dyes. However, further studies are needed to determine its molecular structure. All the data acquired on this unknow marker are reported in the Supplementary Information file (Figure S.5 and S.6).

Finally, Py(HMDS)-GC/MS results ensured the identification of pyrolytic markers of eosin Y (benzoic acid TMS) in French Ink 11, 13 and 15, and of amido naphthol red G in French Ink 13 [56,57]. Instead, the pyrograms obtained for French Ink 2, 7 and 12 were at blank level. No binder or additive was detected for this group of samples.

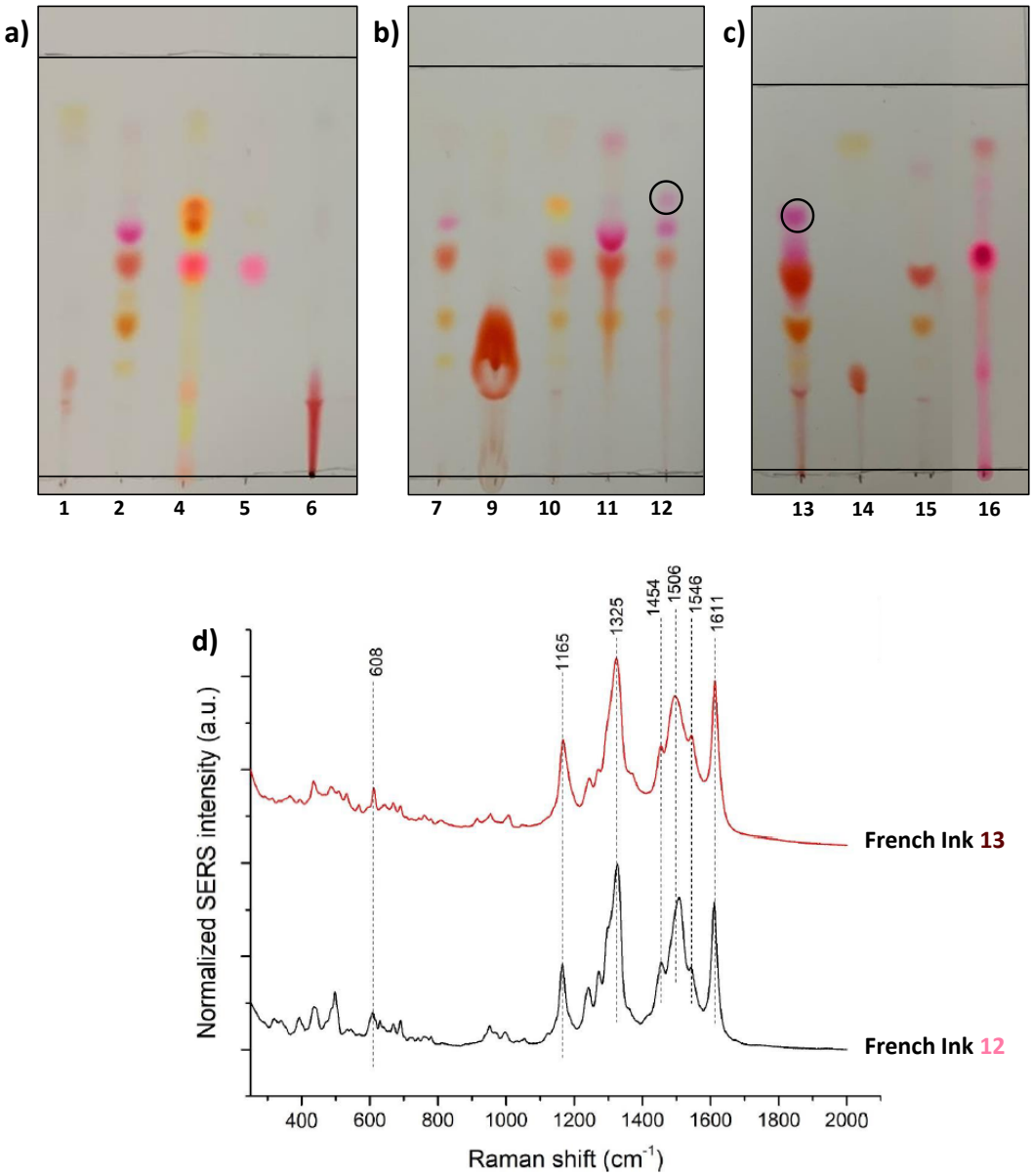


Figure 5: TLC of the French Inks under visible light. a) TLC of French Inks 1, 2, 4, 5 and 6; b) TLC of French Inks 7, 9, 10, 11 and 12; c) TLC of French Inks 13, 14, 15 and 16; d) SERS spectra acquired on Rose Bengal stain (highlighted with black circle).

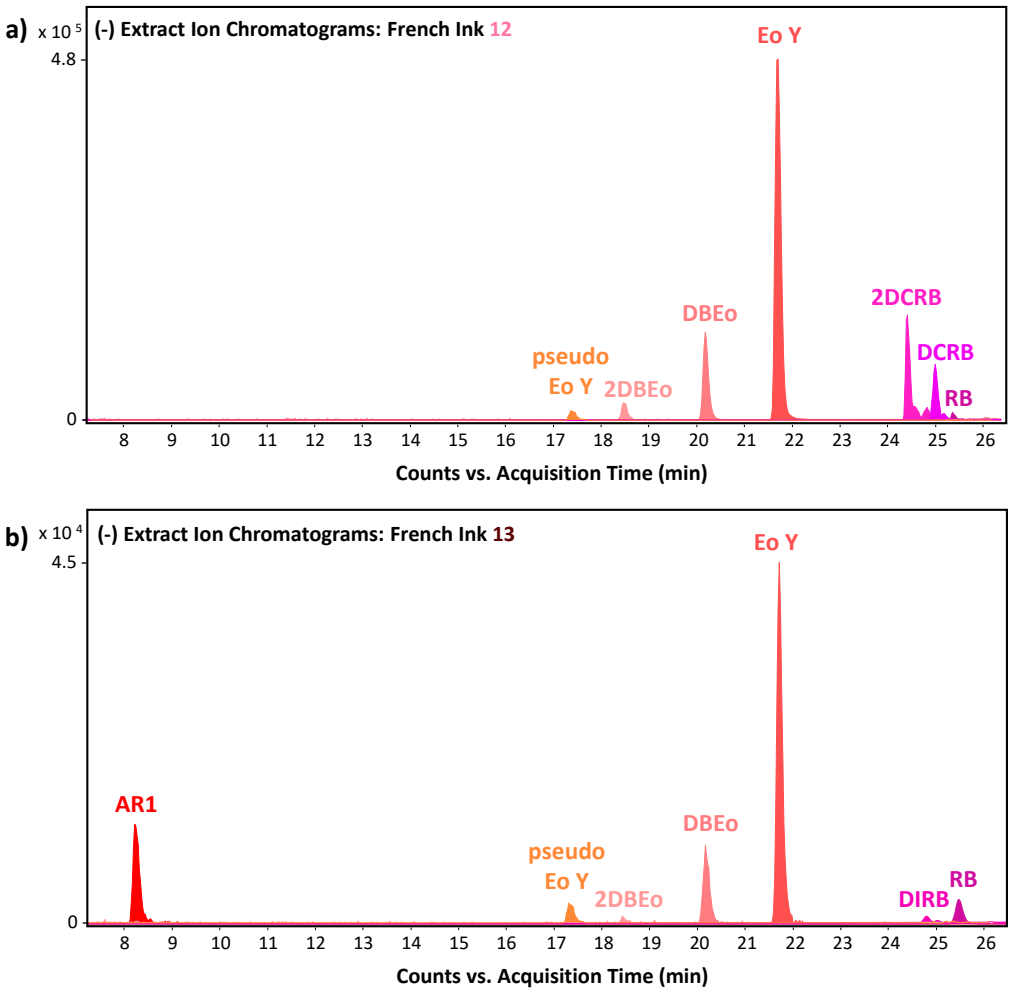


Figure 6: HPLC-ESI-Q-ToF Extract Ion Chromatograms (EIC) obtained by plotting the counts in function of time of the ions corresponding to the molecular ions of the single species detected in the EDTA-DMF extracts of French Ink 12 (Figure a) and French Ink 13 (Figure b). The detected species, highlighted in the chromatograms, are rose Bengal (RB, $C_{20}H_5Cl_4I_4O_5$), dechlorinated rose Bengal (DCRB, $C_{20}H_6Cl_3I_4O_5$), bis-dechlorinated rose Bengal (2DCRB, $C_{20}H_7Cl_2I_4O_5$), deiodinated rose Bengal (DIRB, $C_{20}H_6Cl_4I_3O_5$), eosin Y (Eo, $C_{20}H_8Br_4O_5$), debrominated eosin Y (DBEo, $C_{20}H_9Br_3O_5$), bis-debrominated eosin Y (2DBEo, $C_{20}H_{10}Br_2O_5$) and amido naphthol red G (AR1, $C_{18}H_{14}N_3O_8S_2$). Negative acquisition mode.

### 4.1.3 Rhodamine-based inks

The final subgroup is related to Rhodamine dyes. TLC-SERS experiments confirmed the SERS results and contributed to the identification of *rhodamine B* (RhB) and *eosin Y* (EoY) in French Ink 10, besides the already detected *rhodamine 6G*. HPLC-DAD chromatograms obtained for the EDTA-DMF extracts of French Inks 4,5, 10, and 16 are reported in Figure 7. RhB (19.5 min) and its photo-oxidation products DERhB (17.8 min) and bisDERhB (16.3 min) [52] were detected in all the rhodamine-based inks. HPLC-DAD analysis also enabled us to verify the presence of both rhodamine dyes (RhB and Rh6G) in French Inks 4 and 10, proving that the combination of rhodamine B and 6G was widespread, besides in textiles [43] and paint tubes manufacturing, also in historical ink's formulations. Moreover, *orange II* (AO7, 15.2 min) was determined in French Ink 5, while EoY, AR1, and AR33 were detected in French Ink 10. Both TLC-SERS and HPLC-DAD results were confirmed by HPLC-ESI-Q-ToF analysis, which allowed us to further investigate the composition of these

historical ink's formulations. Positive ionisation mode enabled us to identify first trisDERhB and trisDERh6G (13.7 and 13.5 min, respectively) and Rh (11.5 min) in French Ink 10 (Figure 8b), highlighting an advanced degradation compared to the other rhodamine-based inks [52]. Furthermore, a product related to rhodamine B was detected in French Ink 4, 5 and 16. This unknown molecular marker is characterized by a molecular ion $[M]^+$ at 729.452 m/z, which gives $MS^2$ fragments similar to those of rhodamine B (m/z = 443.235, 399.169) [52]. All the data acquired on this unknow marker are reported in the Supplementary Information file (Figure S.7). Interestingly, the chromatogram acquired in negative ionisation mode features the free acid and esters profile typical of shellac resin [60] in French Inks 4, 5 and 16, as shown in Figure 8.a. Moreover, negative ionisation mode allowed us to identify a methylated analogous of AO7 in French Ink 5 (Figure S.8). The $MS^2$ fragmentation of this species seems to follow the same pathway observed for AO7 (Figure S.9). This suggests the presence of an additional methyl group on the sulfonated ring. The detected molecular marker could be related to Orange 2R (AO8); however, as the standard is not commercially available, this hypothesis was not confirmed.

The presence of shellac resin in French Inks 4 and 5 was further confirmed by Py(HMDS)-GC/MS (Figure S.2), which allowed us to detect butolic acid in its derivatized form [61]. Shellac resin, introduced into Europe as lac dye source in the 12$^{th}$ century and as varnish in the late 16$^{th}$ century [60,62,63], was widespread in ink's recipes to increase the viscosity of the writing materials [64]. Moreover, pyrolytic markers that may be related to rhodamine dyes were detected in French Ink 4, 5 and 16, while the identification of β-naphthol TMS markers in French Ink 5 allow to confirm the presence of orange II [56,57]. Concerning the French Ink 10, the resulting pyrogram was at blank level.

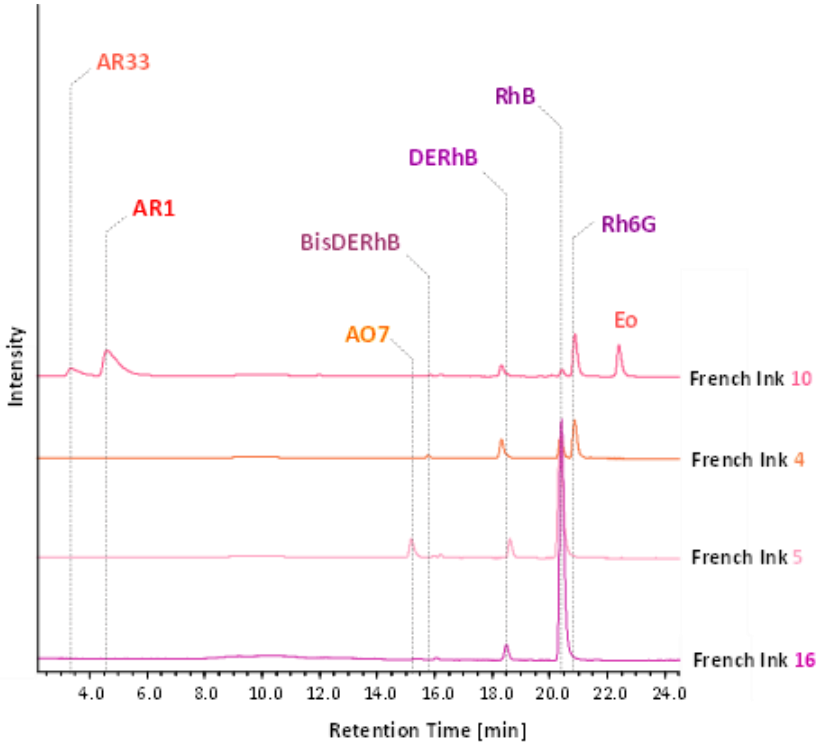


Figure 7: HPLC-DAD chromatograms (500-550 nm) of French Ink 16 (violet profile), 5 (light pink profile), 4 (orange profile) and 6 (pink profile). All chromatograms are presented in the same scale and are stacked for purpose of clarity.

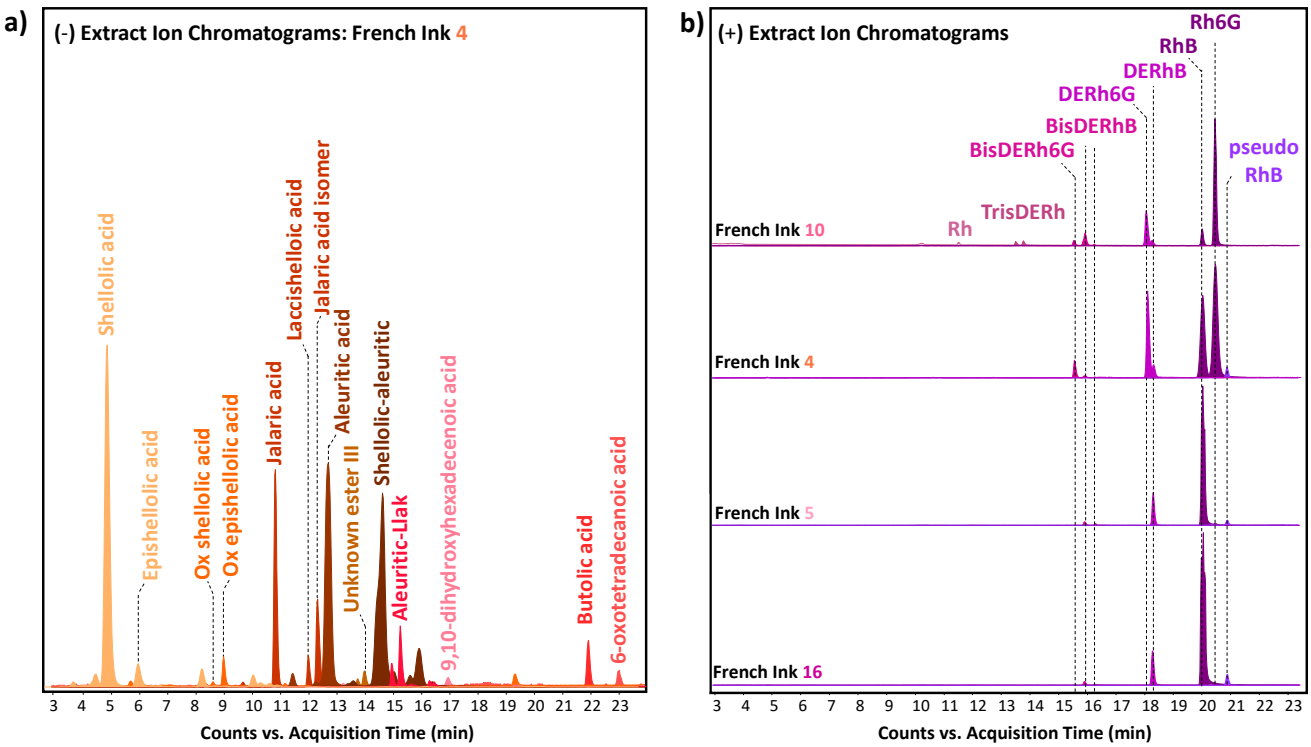


Figure 8: Chromatograms obtained for Rh-based inks. **a)** HPLC-ESI-Q-ToF Extract Ion Chromatograms (EICs) obtained by plotting the counts in function of time of the ions corresponding to the molecular ions of the single species detected in the EDTA-DMF extract of French Ink 4 in negative ionisation mode. The molecular markers highlighted are related to shellac resin. **b)** HPLC-ESI-Q-ToF Extract Ion Chromatograms (EICs) obtained by plotting the counts in function of time of the ions corresponding to the molecular ions of the single species detected in the EDTA-DMF extracts of the French Inks 16, 5, 4, and 10 in positive ionisation mode. The detected species, highlighted in the chromatograms, are rhodamine 6G (Rh 6G, $C_{28}H_{31}N_2O_3^+$), Rhodamine B (Rh B, $C_{28}H_{31}N_2O_3^+$) and its degradation products (DERhB, $C_{26}H_{27}N_2O_3^+$; BisDERhB, $C_{24}H_{23}N_2O_3^+$; TrisDERhB, $C_{22}H_{19}N_2O_3^+$; Rh, $C_{20}H_{15}N_2O_3^+$). All chromatograms are presented in the same scale and are stacked for purpose of clarity.

## 5. Conclusions

To discover the secrets behind the industrial production of writing materials, in the present work a multi-analytical approach was applied for the investigation of an historical ink's collection. The results obtained show that the combination between spectroscopic and chromatographic techniques is a valuable approach to gain information on complex mixture such as writing inks. Trends in historical ink's formulations have been outlined, leading to an overview of the composition of French inks in the late 19$^{th}$ - early 20$^{th}$ century. *Shellac resin*, used as an additive to increase the viscosity of writing materials, was detected in three formulations, while the presence of *gum Arabic*, the binder the most used in historical ink's recipes, was hypothesized only in one ink. Concerning the other inks, no information was obtained for the binder component. This result is in agreement with historical recipes reported in the manuscripts, where the use of binders is not mentioned, e.g. in the details on *alizarine ink* produced by Leonhardi industry, and *Bottger's* recipe of *logwood ink*. The dyes/pigments analysis enabled us to determine that ink's formulations were mainly based on crystal violet, eosin Y, rose Bengal and rhodamine B and 6G. In particular, mixtures of *cotton scarlet* and *crystal violet* were quite characteristic in 19 $^{th}$-20$^{th}$ century ink's formulations. Moreover, *eosin Y* was widespread in ink formulations of the selected historical period, either as a single component or mixed with other dyes such as *amido naphthol red G* and *rose Bengal*. Between all the formulations identified, the one related to rhodamine dyes was the most peculiar. *Rhodamine B* was widely used in 19$^{th}$-20$^{th}$ century inks. However, each manufacturing industry had different secrets on how to tune the composition to obtain specific colours and more performing writing materials. This reflects in ink's formulations in which *rhodamine B* was used alone and in others in which it was combined with complex mixtures of xanthenes and/or hydrazone pigments. Furthermore, new molecular markers for the identification of *methylene blue*, *rose Bengal*, *eosin Y* and *rhodamine B* by liquid chromatography analysis were detected and partially characterised. In addition, TMS-derivatized pyrolytic markers that could be associated to amido naphthol red G, cotton scarlet, orange II, eosin Y and rhodamine dyes were identified in several French Ink formulations. Thus, this paper highlights the suitability of Py(HMDS)-GC/MS methods for the identification of synthetic dyes. Our investigation provides useful information for art technological studies of historical ink collection, expanding the dataset-available in the field of dye degradation products for ink analysis of manuscripts and drawings. Further studies on the historical synthetic routes or possible degradation pathways will be performed, to deepen our understanding of the early days of organic chemistry.

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