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APPLICATION OF ADVANCED METHODOLOGIES TO THE IDENTIFICATION OF NATURAL DYES AND LAKES IN PICTORIAL ARTWORKS

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Tesi di Dottorato di Susanna Marras, matricola 955843

Coordinatore del Dottorato

Prof. Maurizio Selva

Tutore del Dottorando

Prof. Giulio Pojana

Co-tutore del Dottorando

Prof. Renzo Ganzerla
Prof. Antonio Marcomini

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1. GENERAL INTRODUCTION TO THE ISSUES

1.1 INTRODUCTION

Since the dawn of civilization humans have used natural organic matter in order to give vent to their artistic creativity. Naturals extracts (obtained from plants or insects) were directly employed to impart colour to a variety of substrates such as textiles, ointments, cosmetics and artworks. With the development of technology, men learned to produce from these raw materials, real pigments having desired chromatic characteristics; nowadays we called these organic pigments lakes.

Among all the possible topics in the field of art materials, one of the most interesting and fascinating is definitely the study of dyestuffs and pigments. Part of the appeal of this issue, is due to the facts that it reveals the ancient connection between art and chemistry. This relation was well known to past artists since the times the chemistry was called alchemy and was far from the science we know and practice nowadays. The artists of the past were full professionals who must be provided, as well as of artistic talent, of a full mastery of the materials and their properties and of a very good practise in their manipulation.

There are at least three important reasons to develop a study focus on the advancing in the identification of historically used dyestuffs. The first is give an important support to the activity of restorers and conservators who, only having a full knowledge on the artifacts' constituent materials can choose the safest and more appropriate conservative or restorative treatments.

The second is strictly related to the previous one; the study of the chemical composition of lakes could help to better understand the degradation processes that affect organic pigments in a number of ancient and modern paintings (e.g. fading of lakes in paintings).

And third, the identification of individual dyes which came into use at different times and place may contribute to the determination of historical period, provenance or in some case confirm the attribution to a specific artist or school.

Next to these practical reasons, there are many other ones that, even if more speculative, have a fundamental importance in the panorama of artistic, archaeological, historical and ethno-anthropological research. The study of ancient dyes, as well as to increase the knowledge on historical art materials, could in fact provide useful information to the historians, who can know more about the ancient artist's and craftsman's techniques, about the commercial routes for the trade and the distribution of these materials. Since lakes were extremely valuable goods, their identification in an artwork could give information about the social status of the owner. The present thesis reports the results of three years research activity in this fascinating and challenging field.

1.2 CHALLENGES AND TROUBLES

The identification of natural dyes in paintings or in other polychrome artistic objects (e.g. sculptures, archaeological findings, artists' palettes etc.) is a real analytical challenge because of many factors that will be briefly discussed in this introduction.

The first obstacle to their identification is the intrinsic chemical complexity of the related matrix, which is directly related to the nature of the specimens; most artistic and archaeological samples are actually constituted of complex mixtures of many organic and inorganic substances. The second complication is the availability of only very small fragments of pictorial film, which is a direct consequence of the value (artistic, historical and sometimes economical too) of the works of art under investigation.

These issues are common problems for a lot of conservation science analytical tasks, even though they become more critical in the case of dyestuffs analysis in paintings. Furthermore, in this specific case, subsist other difficulties strictly related to the kind of target. Natural dyes are mixture of a lot of substances characterized by different structures (e.g. anthraquinones, flavonoids, etc.) and properties (e.g. hydrophilic, hydrophobic). It follows that a large variety of different chemical compounds may be present in the same sample collected from an historical object. Most of these compounds are barely distinguishable because of their chemical similarity, since some of them are simply structural isomers that just differ for the position of a functional group (positional isomers).

The impossibility to collect a major quantitative of sample is a problem that became much more relevant in this specific case because dyestuffs are usually present at very low concentrations in paintings samples. This fact have two simple explanations: 1) Organic colorants are characterized by a very high tinting power and, as a consequence, very small amounts were sufficient to impart colour to a defined coating or a substrate; lakes were usually applied in very thin layers as a consequence of their employ for the realization of glazes and shading effects.

To further worsen this complex panorama, intervene the ageing processes which may seriously complicate dyestuffs identification by substitution of original compounds with their degradation products. Moreover, natural dyestuffs include in their compositions very labile substances which are easily affected by hydrolytic and photo-oxidative degradation processes (such as the well known fading phenomena that affects many impressionist paintings).

The combination of an efficient extraction procedure, an effective chromatographic separation and the use of a high resolution detector, is therefore required to obtain the selectivity and sensitivity necessary for such research task.

1.4 AIM AND INNOVATIVE ASPECTS

This thesis aimed to give an innovative contribution to the wide and complex field of natural dyes research, with a specific focus on the detection and structural identification of lakes in pictorial artefacts. To achieve this purpose, a systematic chemical-physical characterization of a number of natural dyes traditionally employed for the preparation of lakes has been done.

An additional ambitious task of the study was the compositional comparison of reference lakes in order to identify a set of analytical markers related to specific botanical species or to the making procedures. Another aim was the creation of a spectral database (including UV-VIS, FTIR, MS, MS/MS, and reflectance spectra data) to be used in the routinely analysis of natural dyestuffs. To this end, exhaustive identification sheets have been prepared for all the lake investigated. The adopted multi-analytical approach allowed to collects valuable information and to identify which techniques actually offer significant contribution to the purpose and which not.

The most innovative aspect of this study compared to others in the same field, is the use as reference materials, of real lakes prepared according to ancient procedures. This choice represent an innovative aspect since, disregarding a few exceptions, most studies reported by literature are based on the analysis of plants/insects extracts, commercial dyes or analytical standards. By using such tailored reference materials has been possible to better reproduce the real conditions thus obtaining precious information and developing a more reliable identification strategy.

Another strong point of the present research work was the use of a powerful analytical equipment such as a High Resolution Quadrupole - Time of Flight Mass Spectrometer, which, coupled to a High Performance Liquid Chromatography system, provided the high resolution, selectivity and sensibility required to achieve such challenging analytical task. Thanks to the application of this innovative and performing equipment, a complete fingerprinting of all the substances present in the lakes has been done in this study for the first time. Moreover, a new extraction protocol has been used with the purpose of maximize the information obtainable from the micro-samples. The application of such pre-treatment procedure, which combines a soft de-complexation with an effective solvent extraction of organic components, allowed the identification of both aglycones (dyes) and corresponding glycosides characterizing the different dyeing species considered.

1.4 CONTENTS AND PLAN OF THE WORK

The present work reports the methodology and the results of the analytical characterization of a large set of natural organic lakes synthesized in laboratory according to ancient recipes. The planned approach involved three steps: 1) the extraction and preparation of lakes starting from raw materials according to ancient treatises; 2) the analysis of the above mentioned pigments by means of High Performance Liquid Chromatography coupled to Diode Array Detector and Quadrupole-Time of Flight Mass Spectrometry; 3) the validation of the methodology by application to real samples of paintings from various ages and origins. Concurrently, a complete characterization of lakes by means of spectroscopic and microscopy techniques has been carried out resulting in the creation of a complete reference spectra database.

More than 600 samples were analyzed throughout the whole research period, including: 1) a set composed of pure lakes specifically synthesized to be used as reference materials; 2) a set of the same lakes subjected to artificial aging by irradiation with UV- VIS light in a climatic chamber at controlled temperature and humidity; 3) a set collected from reference paint coatings realized with the above lakes dispersed in both oleic (linseed oil tablet) and protein binder (egg tempera tablet); 4) a set of real samples collected from Italian and Spanish paintings.

The thesis starts with an introductory section that briefly explains what the lakes are and why their identification in paintings is so important (Chap. 2). In this introduction chapter the analytical techniques and the theoretical principles on which the former are founded are also presented and described (Chap.3). The dissertation continues with the experimental section that describes all materials, techniques and procedures used in the different phases of the laboratory activity, including the preparation of reference materials (Chap. 4) and the preparation/extraction procedure and the analysis of samples (Chap. 5). The next chapters are reserved to the discussion of the results with a focus on original findings and future perspectives. This section is organized into four chapter addressed to the discussion of anthraquinones (Chap. 6), flavonoids (Chap. 7), indigoids (Chap. 8) and other dyes (Chap. 9) respectively. It was decided to deal separately with the results of each dye species in order to create a sort of ID sheet for each of them. For this reason, each chapter present an internal division in paragraphs corresponding to the various species considered in the study. All MS and MS/MS spectra discussed in chapters 6-9 are inserted as Annex at the end of the thesis. A selection of real cases investigated in the course of the research activities is discussed in the following chapter (Chap. 10). A complete record of all the experimented recipes with useful additions and photos caught during the making of, is provided at the end of the thesis (Annex II).

2. DYES, LAKES, PIGMENTS: OVERVIEW ON THE WORLD OF COLOR

2.1. DYE, PIGMENT OR LAKE?

Talking about colouring materials there is a lot of confusion concerning the meaning of the terms used to correctly identify them. Is therefore important open a brief parenthesis on this issue, in order to clarify once for all, the distinction among a dye, a pigment and a lake.

The term **pigment** identifies a fine solid coloured powder, insoluble in the dispersing medium, with which forms a differently pastry cream that manifests covering properties even if applied in very thin layers. Due to these characteristics, pigments are particularly adapted to be used as pictorial colouring matter. Since they do not exhibit adhesion properties, to be applied must be mixed to a binder (organic or inorganic).

On the contrary **dyes** are generally water soluble, transparent substances, able to impart colour to others by inclusion, adsorption or through the formation of specific chemical bonds. According to these features their main application is in the sector of materials dying (e.g. textiles, paper, plastics, food etc).

The majority of traditional pigments historically used in the artistic field was inorganic (natural or artificial) but there is a conspicuous group of organic pigments that reached a great importance and diffusion as well. They were obtained from natural dyes extracted from vegetal or animal species.

With the exception of a few insoluble-type ones, the organic dyes have to be converted into pigments by means of mordanting processes (precipitation of insoluble pigments by formation of metal-dyestuff complexes) or adsorption into inert powders, to be use as painting material. This type of artificial organic pigments takes the name of **lakes**.

Chemically a lake is therefore a coordination complex between an opportune organic dye and a metal cation. Some of most famous lakes have been extensively investigated in order to understand the chemical structure of the complexes. An example referred to madder lake is shown in Fig.2.1.

Nowadays a wide number of transition metals in the form of salt are used for the fabrication of synthetic organic pigments, but in ancient times the most common mordant was aluminium in the form of potash alum (Aluminium potassium sulphate dodecahydrate, $KAl(SO_4)_2 \cdot 12H_2O$) or alumina (aluminium oxide, Al_2O_3). In some case iron, copper tin have been used.

A wide number of well documented procedures for the fabrication of lakes is available from old literature. Until 19th century most receipts prescribed to precipitate the lake from the solution containing the dyestuffs by adding alum and alkali. The reaction between potash alum and an alkali forms a type of substrate that, to simplify, is normally described as hydrated alumina. Actually its exact nature it has not been explained yet. It seems not correspond to any of the kown crystalline aluminium (oxy)hydroxides or anhydrous oxides. It is really complex and difficult to characterise because is partly amorphous and strongly depending on a number of factors such as temperature, pH, nature of the base (K_2CO_3, Na_2CO_3, $CaCO_3$) and even the order of addition of the potash alum and the alkali. All these factors have moreover a direct effect on the composition and aspect of the resulting lake.

Fig. 2.1 - Complexation sites in alizarin molecule (a,b) and proposed structure for a alizarin-aluminum complex

Lakes are usually characterized by high brilliancy, a glossy or translucent aspect and sometimes unusual colours (using mineral pigments purple or violet hues can be obtained only by mixing or superposing red and blue pigments). For these reasons they were particularly appreciated by artist and could be very prized. To better exploit their quality artists used to apply lakes as glazing pigments in order to give a bright final touch to their paintings and obtain effects not achievable with available mineral pigments. Although lakes can be used in a relatively wide range of binding media, were more frequently applied in oil, or in oil-resin binders in order to further enhance the translucency.

2.2 HISTORICAL OVERVIEW ON LAKES

Lakes have a long history of usage. For millennia, humans have employed colouring matters supplied from nature to adorn their bodies, to give colours to their dresses, to pay tribute to their gods and, above all, to express their thoughts and creativity by painting. History of natural lakes is strictly connected with that of natural tinctorial dyes, that have been used used for more than 4000 years until the advent of synthetic dyes in the last one and a half centuries.

The first evidence of coloured substances application dates back to approx 400.000 years ago with the primordial cave paintings. Anthropologists claim that prehistoric men used to employ natural colouring matters (both mineral and vegetal), as well as to realize paints, and also to dye their body with rituals purposes during hunting or to scare enemies.

This practice was maintained by many ancient populations. Is a well know fact that in the Iceni tribe, the ancient inhabitants of Britannia, the warriors were used to paint their faces with the blue woad dye before going into battle, even if this fact is currently questioned by several historians who attribute the belief to an incorrect translation[1]. Soon, with the beginning of the textile era, early humans learnt to exploit natural colours to dye fibres. From historical sources and from archaeological investigations results effectively that the use of natural dyes for tinctorial purpose, was well established in the various ancient cultures spreads all over the world such as that of China, India, Mesopotamia, Egypt and Central South America.

The invention of lakes is attributed to Ancient Egyptians. Just like many other civilizations, they were familiar with the dyeing properties of some natural species, but have been the firsts to employ such dyes for artistic purposes. They developed alchemic processes to obtain insoluble pigments from coloured plant extracts by fixing the dyes onto colourless inert powders such as hydrated clays. They habitually dyed their clothing and other materials with a number of documented natural organic dyes and were so able in the technique that left detailed descriptions of the procedures. Organic dyestuffs were also used to prepare cosmetics.

In ancient Rome dyes were as valuable as gold. The secrets of natural colours were kept by priest and craftsmen and a sort of licence was required to manage some special dyestuffs. Wear dyeing clothes was sometimes a sign of high social status. For example is a well known fact that in Roman culture only the emperor and the high priests could wear purple garments, vests dyed with a prized dye extracted from murex shells, since it was a symbol of

[1] The sentence "Omnes vero se Britanni vitro inficiunt, quod caeruleum efficit colorem", written by Julius Caesar when

wealth and power. It was so precious and expansive that even the amount of purple present in the vests was a measure of the individual's importance (in the Arab countries and in China the same importance of the purple was attributed to saffron; only the Chinese emperor could wear saffron coloured vests).
Purple was the most important dyestuff imported by the Romans, probably from Phoenicians who already known in the former 15th century BC. They were famous masters in the production and application of this precious dye insomuch as the most famous of the antiquity was the Tyrian purple. Even if the dying of textiles was the most developed application of dyestuffs in the Roman culture, documental and archaeological evidences testify the consolidated use of organic pigments obtained by absorbing natural dyes on clay or diatomite substrates. One of the plants used to this purpose was the madder, native of Greece and used as dye source perhaps as early as classical times.

The mediaeval artist palette was characterised by the use of bright colours, mainly constituted by mineral powders such as hearths and metal oxides. In the Middle Age the only known red lakes were obtained from the lac insect (Laccifer lacca), very common and largely used also for the production of shellac, from the oriental brazil wood and from the Madder roots. Was also fairly common practice to extract the dyes from dyeing industry scraps, habits condemned by Cennino Cennini in his "the art book" as it led to obtain poor quality lakes.
The main sector of use of organic pigments in the Middle Ages was that of miniature. Thanks to recent studies the use of very unusual and creative sources of colorants in these artefacts is being discovered. This habit is probably due to the reduced dimension of the illustrations that allowed to apply also very expensive pigments, and to the fact that, since illuminated manuscripts were guarded in protected sites, the artists could use pigments usually avoid because of their poor light fastness.

At that time, the manufacturing technology of lakes was still primordial. With the pass of time it improved considerably but in a slow and gradual way so that in the fifteenth century it was still considered a novelty and required special skills. This character of exclusivity explained their high price with respect to other pigments to such an extent their use was clearly mentioned in the contracts between painter and customer. For the same reason sometimes the customers pretended evidence that the lakes they were going to purchase were genuine. To this purpose, simple tests useful for the identification of fakes were developed. The demand of organic pigments gradually grew in the following centuries, and between the Fourteenth and Nineteenth centuries they were frequently employed by almost all European painters. Purple, red and yellow lakes were particularly appreciated and were

usually applied as thin, transparent glazes above layers of inorganic pigments to create transparency or depth effects, or in mixture with other pigments to obtain special chromatic tones (e.g. mixture of lead white and red cochineal lake gave precious pink hues).
Even though green is together with yellow one of the most spread colours in nature, there are very few green natural dyestuffs. Artists used to obtain green tones by mixing blue and yellow dyes. Since most blue dyestuffs are more stable than yellow ones, green colours are often prone to change into blue with the pass of time. This is the main reason why there are so many examples of historical paintings with blue shades, where originally green areas were present.

The golden age of natural lakes has probably been the Renaissance period with the increasing of the demand of new colour matters for the dyeing industry. The research of new dyestuff to satisfy the needing of coloured clothes the dying activity has been a driving force for lakes production because was combine to the investigation on procedures to use the same rich dyes in artistic sector. Documental sources testify that in high Renaissance in Italy Lac was the third most expensive pigment (after gold and Ultramarine).

The discovery of the new American continent in 1492 gave a significant additional contribute to the colour world since it led to the introduction of new natural dyes (e.g. cochineal) or new sources for the supply of well known ones (e.g. brazil, logwood). Actually the trade of dyes, together with that of metals, from the new world made the fortune of the Spanish and Portuguese colonizers.

The time period between the second half of Seventeenth century and the first half of the Eighteenth century has been marked by a very peculiar figure in the field of lake manufacture; the painter Vittore Ghislandi known has Fra Galgario. He was famous not so much for his paintings but for the production of stunning high quality red lakes (probably made using American cochineal) very sought-after by his contemporary artists.
In 19_{th} century an unusual pigment called Indian yellow was introduced in Europe achieving resounding success among the experienced artists of the time. It was an intense yellow dye, used in India since the 15_{th} century, obtained from urine of cow exclusively fed with mango leaves. It was banned in the 20_{th} century due to the diseases caused by the practise to the poor cows.
In 1856 William Perkins serendipitously discovered the mauve, the first aniline dye, so beginning the new era of synthetic colours. He was a 18 year old chemistry student when, in a attempt to produce quinine, he obtained a strange murky residue that 15 days later, at the moment to be throw away as the forgotten evidence of an experimental failure, reveals itself as a beautiful violet colour. Perkins wrote about the episode: "was about to throw a certain

residue away when I thought it might be interesting. The solution of it resulted in a strangely beautiful colour." The new artificial colours, thanks to low production costs and the possibility to achieve all the desired shades, ended up almost completely replacing not only the natural dyes but also the inorganic pigments, to such an extent that nowadays almost all the traded colour tubes are made of synthetic dyes.

2.3 PRINCIPLES OF COLOUR TEORY

The colour is a very complex phenomenon that involves both the person who perceive the chromatic sensation and the object that is perceived as coloured. The colour of dyes and pigments depend firstly to the wavelength of the light absorbed by its constituent compounds; if no light have been absorbed a substance appear colourless. In case of absorption of one on more light radiations, the eyes perceive the substance as coloured. The observed colour results from the subtraction of the absorbed light that means in other words that the chromatic perception correspond to the complementary colour. In order to have a substance appearing as coloured to human eyes, the absorbed radiations must belong at least partly to the visible range of the electromagnetic spectrum that is comprised between approximately 400 to 700 nm as wavelength. The following table resumes the correlation exiting between the absorbed radiation and the observed colour (called complementary colour). This table refers to single radiation absorption but of course the same colour perception can be obtained with the simultaneous absorption of two or more different wavelengths.

WAVELENGTHS	ABSORBED COLOUR	OBSERVED COLOUR
< 400 nm	ultraviolet	none
400-435 nm	violet	yellow-green
435-480 nm	blue	yellow
480-490 nm	blue-green	orange
490-500 nm	green-blue	red
500-560 nm	green	purple
560-580 nm	yellow-green	violet
580-600 nm	yellow	blue
600-650 nm	orange	Blue-green
650-750 nm	red	green-blue

Tab. 2.1 - correlation between absorbed and observe radiation

Various theories have been elaborated to explain why a molecule is coloured. One of the first and most accredited, is that developed by the German chemist Otto Witt in 1876, later refined by Dilthey and Witzinger in 1928.

Witt's theory states that an organic substance to be coloured must contain at least one chromophore group, and to be able to colour a substrate at least one auxochrome group. Molecules containing one or more chromophors are called cromogens.
The cromophores (from the greek words chroma and pherein which means respectively colour and to bear) are functional groups responsible for the colour. They are commonly withdrawing groups. The intensity of the colour increases with the increase in the number of chromophores in a molecule. The effect is particularly marked if the chromophores are conjugated; for example molecule containing a single C=C are colourless but molecoles with 6 (e.g $CH_3CH=CH)_6CH_3$) or 12 (e.g $CH_3CH=CH)_{12}CH_3$) conjugated double bonds are respectively yellow and red. ome of the mostimportant chromophoric groups are:

The auxochromes (from the greek auxein which means to increase) are functions that intensify the colour of the molecules increasing by shifting the absorbed wavelength value to a longer one value (bathochromic effect). The auxochromic groups also have the function to make the molecule soluble (or to increase its solubility) and, as said before, to convert them in dye. Sometimes is directly responsible of the colour because of the shifting of this absorption value from the ultraviolet area to the visible one and making coloured a previously uncoloured substance. The principal auxochromic groups are:

2.4 CLASSIFICATION OF DYES

The earliest compendiums of natural dye were simples alphabetical order list, sometimes organized according to the related botanical species. As time goes by, with the growing importance of dyeing activity in the modern society, several classification systems have been conceived for a better comprehension of the dyes. Some of them have been specifically designed for natural compound (e.g. classification according to the biologic origin), whereas others can be applied both to naturals and synthetics. The two most important, because of their utility and efficiency, are the technical (or tinctorial) classification system, which identifies seven groups depending on the way they are applied, and the chemical one, which classifies the dyes on the basis of the chemical structures of their main cromogens. Fairly widespread is as well the system that simply divides the dyes on the basis of their color in red-orange dyes, yellow dyes, blue-violet dyes and brown-black dyes. The absence of an independent green dyes group is probably due to the fact that only few natural organic dyestuffs are truly green. This intuitive method is partially related to the chemical one since a specific dyeing compound family is generally correlated with specific hues.
According to the technical classification organic dyes can be divided as follow:

1. **MORDANT DYES -** Mordant dyes are dyestuffs which require the addition of a bridging unit to be applied since they do not present affinity with the fibres and are unable to fix to it in a stable way. The textile must be previously treated with a mordanting agent (a metal salt or a suitable coordinating complex forming agents) that remains attached to the fibres by coordination processes (mainly by chelation). The dye form itself a coordinating complex with the metal remaining in this way anchored to the fibre. A mordant dye needs to have electron donating groups capable of forming a complex with the transition metal salt. (e.g., madder, fustic, persian, berries, kermes, cochineal etc).

Fig. 2.2 schematic representation of the dye-fibre linking process

2. **DIRECT OR SUBSTANTIVE DYES -** The dyes belonging to this group have a chemical affinity with the fibre and consequently they do not require a specific treatment to be stably fixed to it. A direct dye links to the fibres trough a strong primary bond, usually a ionic one, based on the electrostatic attraction between opposite charges. Typical examples of direct dyes are curcuma and saffron.

Fig. 2.3 - schematic representation of the dye-fibre linking process

3. **VAT DYES -** The vat dyes are water insoluble substances that can be converted into a soluble form, usually colourless (leuco form), by means of alkaline reduction. In this way the fibres could be easily impregnated with the dyeing solution. The final colour is developed directly in the fibres in a second step by exposure to air and consequent oxidation of the leuco form to the colour one. The name "vat dyes" derives from the wooden vat formerly used as dyeing bath. Typical examples of direct dyes are indigo and purple.

4. **ACID DYES -** Acid dyes are water soluble anionic dyes which possess affinity for amphoteric fibres such as protein fibres (e.g. sink, wool), nylon and modified acrylics. Chemically they are sodium (less often ammonium) salt which containing either sulphonic, carboxylic or phenol group (s). They require the use of an acidic medium (vinegar, acetic or sulphuric acid) to be efficiently applied. In fact in acidic conditions, the amino groups of the fibres are protonated to give a positive charge ($-NH_3^+$) which can interact with the anionic dye forming electrovalent bonds and allowing in this way the fixing. The colour stability is increased by the contemporary formation of Van-der-Waals bonds, dipolar bonds and hydrogen bonds. They usually have better light fastness than basic dyes. (e.g. saffron)

5. **BASIC DYES** - Basic dyes are water insoluble compound in their original form (base) but can be made soluble by conversion into their relative salts (usually chlorides). They are also called cationic dyes because of the positive charge yield by their chromophores in the ionic form. From a chemical point of view they present amino or alkylamino groups as auxochromes which confer to the molecules a particular affinity to anionic or negatively charged materials. The mechanism of attaching to the fibres is the same of the acid ones with the obvious difference that, since they are

positive charged, they forms electrovalent bonds in the anionic sites of the substrate. They are characterized by high brightness and strength but also by a poor light fastness. (e.g. berberine)

6. **REACTIVE DYES** - The reactive dyes contain functional groups able to interact directly with the fibres by creating irreversible bonds between dyes molecule and the textile fibres. They are mostly applied on cellulosic material (e.g. cotton and lined fabric, paper etc.) but are very effective on wool and nylon (under weakly acid conditions) as well. Despite their high light fastness reactive dyes are less used compared to other types of dyestuffs because of their tendency to hydrolyse.

7. **SULFUR DYES -** Sulfur dyes are synthetic organic substantive dyes for cellulosic fibres such as cotton, linen and jute. These dyes are water insoluble but can be converted in a soluble form by treating with a weak alkaline solution of sodium sulfide or sodium hydrosulfite (also called sodium dithionite) which constitutes the dyeing bath. Under these conditions the dissolved dyes are absorbed by the fibre in their soluble but colourless form and then oxidized back to their original insoluble form by exposure to air or introduction of suitable oxidizing agents.

8. **DISPERSE DYES -** Disperse dyes are non-ionic substances with low solubility in water and relatively low molecular masses (in the range of 400 – 600 Dalton). Due to these characteristics they can be efficiently applied on to hydrophobic synthetic fibre such as polyester, nylon and acetate. The low molecular weight is important to avoid an efficient diffusion into textiles. They are azobenzene, anthraquinone or diphenylamine molecules with nitro, amine, hydroxyl or, more in general, no water solubilising groups attached to it. Due to their hydrofobicity the disperse dyes need a dispersing agent to be brought into solution or better into a stable dispersion. The dyeing is achieved via micelles formation and subsequent release of the dyestuff into the fibres.

Fig. 2.1 - Disperse dyeing mechanism

The technical classification is particularly appreciated and used by industrialists and dyers, in other words by manufacturers and customers. Even though very useful from a practical point of view, this classification system is not so effective to a speculative/didactic one. In effect it presents a number of superimposition due to the fact that a lot of dyestuffs can be applied with more than one dyeing technique.

The scientific community prefers the chemical approach of classifying dyes according to the type of chromophores. According to this system a first subdivision must be done between natural and synthetic a dyestuff, that is, on the base of their origin. Since the topic of the present research is the study of the traditional dyes used in paintings, and since before the second half of 19_{th} century the dyes were obtained exclusively from natural sources, a detailed discussion on synthetic dyestuffs has been omitted. Anyway, for sake of completeness, the current chemical classification of synthetic dyes is shown in Tab. 2.

As regards the natural dyestuffs, even though several proposed classification characterized by a greater or lesser degree of clustering exists, they were generally classified into 7 groups: antraquinones, flavonoids, indigoids, naphthoquinonic, carotenoids, xanthonoids and tannins.

The next section focuses on these dyes families, illustrating the main features and, for each category, providing a complete description of the historical dyes used for pigments production.

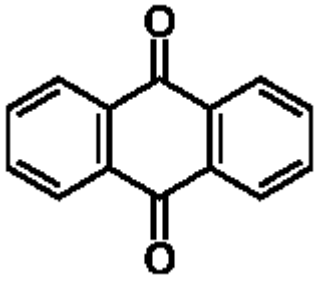
▸ Quinone-imine dyes, derivatives of quinone	▸ Quinone-imine dyes, derivatives of quinone
▸ Arylmethane dyes ‣ Diarylmethane dyes, based on diphenyl methane ‣ Triarylmethane dyes, based on triphenyl methane	▸ Azin dyes ‣ Eurhodin dyes ‣ Safranin dyes, derivatives of safranin
▸ Azo dyes, based on a azo structure	▸ Xanthene dyes, derived from xanthene
▸ Cyanine dyes, derivatives of phthalocyanine	▸ Indophenol dyes, derivatives of indophenol
▸ Diazonium dyes, based on diazonium salts	▸ Oxazin dyes, derivatives of oxazin
▸ Nitro dyes, based on the nitro functional group	▸ Oxazone dyes, derivatives of oxazone
▸ Nitroso dyes, based on nitroso functional group	▸ Thiazin dyes, derivatives of thiazin
▸ Phthalocyanine dyes, derivatives of phthalocyanine	▸ Thiazole dyes, derivatives of thiazole
▸ Rhodamine dyes, derivatives of rhodamine	▸ Fluorene dyes, derivatives of fluorene
▸ Pyronin dyes	▸ Acridine dyes, derivates of acridine

Tab. 2.2 - chemical classification of syntetic dyes

2.5 CHEMISTRY OF NATURAL ORGANIC DYES

From a chemical point of view natural colors can be classified on the basis of their molecular structure in: antraquinones, flavonoids, indigoids, naphthoquinonic, carotenoids, xanthonoids and tannins. In the following paragraphs the main features of the different category are illustrated and, for each category, a complete description of the respective dyes examined in this study is provided.

2.5.1 ANTHRAQUINONES



Antraquinones are derivatives of the 9,10-anthracenedione molecule (anthraquinone) which basic structure is characterized by an anthracene core which presents a quinonic group. The different anthraquinonic compounds differ in the nature and positions of substituent groups. They can be divided into two wide classes, alizarin and emodin types, depending on their biosynthetic pathway and substitution pattern.

The alizarin type anthraquinones have only one of the rings unsubstituted and are formed via chorismate/δ-succinylbenzoic acid pathway. They are mainly found in Rubiaceae plants (such as Rubia, Morinda, Galium and Cinchona). The emodin type anthraquinones have instead both rings substituted and are formed through the polyketide pathway (acetate-malonate pathway).
From the technical point of view can be mordant, acid or vat dyes although the great majority belongs to first category. In the vat and acid applications of anthraquinonic dyes, tends to predominate the violet, blue and green hues. The mordant ones use to be polygenetic, that means that the resulting lake color can vary considerably depending on the mordanting agent (in this case from red to purple or blue).

According to the molecular structure, the mordant-dye complex is established by means of the carbonyl group and the adjacent phenolic group. The most famous antraquinonic dye is the alizarin which can form crimson red lakes with calcium, barium and strontium, pink-red lakes with aluminium, brown-violet lakes with chrome and copper, and black-violet lakes with mercury and iron III. All the main red colorants obtained from vegetal and animal sources belong to this class. Antraquinonic dyes are generally characterized by a good stability to light and to washing.

MADDER

Other names: robbia, garanza, meekrap, garance, kraoo, färberröte, al-izari, alzan, meede, mee, rote, roza di fiandra, rubia major, varantina, warance, krapfo, warantia, barentia

Madder lake is one of the oldest and most famous vegetal pigment of the antiquity. It was prepared starting from the roots of Rubiaceae family plants. The two most important (for history and diffusion) are Rubia tinctorum L. (Madder) and Rubia peregrine L. (Wild madder), which grow from Mediterranean Europe to Asia. Others remarkable species of the genus Rubia are Rubia cordifolia (Munjeet) and Rubia sikkimensis, both from India and Southeastern Asia, and Rubia akane, endemic of Japan

Fig. 2.5 Rubia tinctorium L (a) Rubia peregrine L (b) Rubia cordifolia (c)

In addition the dyestuff could be obtained also from the species Galium verum L. (Lady's bedstraw), Galium mollugo L. (Hedge bedstraw) and Relbunium hypocarpium L. Belonging to the genus Galium and Relbunium respectively.

Fig. 2.6 Galium verum L (a) Galium mollugo L (b) Relbunium hypocarpium L (c)

Among all the rubiacee the specie Rubia tinctorum is the most spread and historically important. It is native of Southern Europe and it was well known to the ancient Greeks, who called it erythrodanon, and to the Romans with the name rubia. Plinio talked of this plant not only for the dyeng of texiles but also as pigment subsequently to the absorption on inert materials as clayey earths, white clay, or powdered cuttlefish bones. Madder details on painting were discovered during the excavations of Pompei and in the baths of the Roman emperor Titus. The finding of madder in the marriage certificate of the empress Theophano dating from 972, demonstrated its employ also in the byzantine culture.
The plant was cultivated in France in the VI century, later Carlo Magno endorsed its cultivation in the whole empire with an official agricultural reform. In the Italy it was cultivated in Lombardy, Romagna and in the Siena area. Until the XVIII century the

Orientals used to dye linen and cotton cloth a red coloring substance which called "Turkish red". In the occidental word was instead preferable used to dye animal fibers as wool or silk. The synthetic dye (alizarin) was synthesized for the first time in 1868 with a consequent large scale production of the industrial dyes and demise of the madder crops.

In order to obtain the dye the madder roots were traditionally heated (either in a dry process or with steam) and then stored for months or years. This was important to allow the release of the aglycones, responsible of the dyeing, by hydrolyzing the glycosides by thermal, hydrolytic, or enzymatic processes. The storage causes also the formation of purpurin and xanthopurpurin by decarboxylation of pseudopurpurin and munjistin respectively. The dye was really As fabric dye it was used with alum mordant to obtain a red tone, with iron to obtain violet tones, with chromium to obtain brown ones. The Madder lakes, characterized by a great light fastness, were mainly complexes of the anthraquinones dyes with aluminum cations, adsorbed on amorphous alumina.

Madder lake is constituted of a mixture of anthraquinonic compounds whose percentage distribution depends on many factors such as origin, plant species and dye extraction procedure. The quantitative main compounds are alizarin (1,2-dihydroxyanthraquinone) and purpurin (1,2,4-trihydroxyanthraquinone) followed by pseudopurpurin (1,2,4- trihydroxy-3-carboxyanthraquinone), lucidin (1,3-dihydroxy-2-hydroxymethylanthraquinone), xanthopurpurin (1,3-dihydroxyanthraquinone), rubiadin (1,3-dihydroxy-2-methylanthraquinone) and munjistin (1,3-dihydroxy-9,10-dioxoanthracene-2-carboxylic acid). It should be emphasized that in the madder obtained from Rubia peregrina (wild madder) the main component is the rubiadin and that the alizarin is almost absent while in that obtained from Rubia cordifolia the principal dye is the munjistin.

Fig. 2.7 structural formulas of the main aglycons in Madder extracts

KERMES

Other names: crimson; lac; carmine; chinese lake; cimatura; crimson lake; florentine lake; grana; Hamburgh lake; purple lake; roman lake; scarlet lake; venetian lake; , carminic acid, scarlatum, chermes

Kermes is a red dyestuff derived from the female specimens of various Kermesidae species. The most important is Kermes vermilio Planchon which is indigenous to the Mediterranean area with a particular diffusion in Spain, southern France, Italy and Crete. The insect found its perfect habitat on a Mediterranean tree, the Quercus coccifera L., also call for this reason kermes or scarlet oak. Another documented source of kermes dye is the specie Kermes ballotae even though to a certainly lesser extent. Some historical source mentioned also the use of Coccus illicis L., an insets which lives oak Quercus ilex L. but the facts demonstrated that is impossible obtain a dyestuff from this insect. The eggs of kermes insects, which have the appearance of red grains, in the antiquity were confused with vegetal berries. This is testified by the many names referring to berries or grain also used to identify kermes. For example Theophrastus and Dioscorides refer to kermes with the word coccus ('κοκκος'), which means berry.

Fig. 2.8 ***Kermes vermilio Planchon***

The extraction and preparation of the dyestuff was historically similar to other scale insect dyes; The grains were harvested, treated with an acid solution (e.g. vinegar) and dried. The coloring component was later extracted from the dry material. It was a very common practice to extract the dyestuff from previously dyed fabrics. The resultant lakes were identified with names referring to this procedure such as cimatura (Italian), vloken (German) or bourre (French) lakes. Is a polygenetic dye and its color can vary depending from the used mordant: with aluminum are obtained crimson red hues, with tin scarlet red ones and with iron purple shades.

The first description of the insect dates back to the XVI century. Documentary evidences indicate that kermes was known and used in Europe from Roman times, or even before. At the beginning kermes was used to dye silk as a substitute of tyrian purple, much more expensive, while later became the principal red dye for the dyeing of Venetian textiles under the commercial name of "scarlatto veneziano" or "rosso grana". After a big fortune and employment kermes was gradually almost completely replaced by the American cochineal available starting from the 1540s. Another cause of abandon of such lake, even if secondary,

was the decrease of the insects, nowadays nearly extinct, as a result of drastic Europe deforestation and consequent lost of their natural habitat. The main component of the kermes extract are kermesic acid (>75%) and flavokermesic acid (10–25%).

Fig. 2.9 structural formulas of the main aglycons in Kermes extract

LAC DYE

Other names: Indian lake, shellac

Is a red dye obtained from the waxen-resinous secretion produced by various species of scale insects (Coccoidea**)** belonging to Kerridae family. The principal one is Kerria lacca Kerr (also known with the name Coccus lacca or Laccifer lacca) indigenous to India, south China, Southeast Asia, Thailand, Philippines and Sumatra. These insects live in various plants mainly belonging to the Croton and Ficus species and the quality of the dyestuff depend in part on the host plant. In the branches of these host trees, the females form with their larvae colonies that can get to count from 50 to 100 larvae per cm^2 producing a brown red exudation.

Fig. 2.10 Kermes vermilio Planchon

This exudation is mainly collected to produce shellac and gum but was also subjected to extraction (with hot water or with a sodium carbonate solution) in order to obtain the dyestuff. The Lac dye was well know to the ancient Egyptians and Persians who in the XV e XVI century use the dye to decorate fabrics and carpets. In Europe not had the same fortune as fabric dyestuff being used during a short period from about 1790 to 1870 particularly in England. The lake was firstly produced in India, China and in many other region of Southeast Asia. It began to spread in Europe in the first half of XIII century thanks to Catalans and Provencals.

Is often mentioned as primary red lake used for easel painting in fifteenth century Italy. With the pass of time was subjected to the same end of the kermes, replaced to the American cochineal. The major constituents are the laccaic acids as well as erythrolaccin, isoerythrolaccin and deoxyerythrolaccin. The name laccaic acids include several anthraquinone compounds having very similar chemical structures and labelled with alphabetical letters from A to F.

COCHINEAL

Other names: crimson, grana, scarlatto veneziano, cochineal

Is a red dye obtained from the dye bodies of the female of some insect belonging to the family of Dactylopiidae (coccoidea). The most important is the Dactylopius coccus costa or Coccus cacti L. (american cochineal), who lives in the cactus or prickly pear plants (Opuntia coccinellifera). Other two important source of the dye are the coccids Porphyrophora polonica L. (Polish cochineal) extensively used in central and northern Europe to dye silk in XV and XVI century, and the Porphyrophora hamelii Brandt (Armenian cochineal) used in Armenia, Turkey and Iran from the 8 B.C.

a **b** **c**

Fig. 2.11 - Dactylopius coccus costa (a) Porphyrophora polonica (b) Porphyrophora hamelii (c)

The cochineal dye is one of the most light and heat stable of all the colorants and is more stable than many synthetic food colors.

This colorant originating from Mexico and Guatemala was used to dye textiles since from the pre-Colombian populations (Incas, Maya and Aztecs). Since the antiquity the cochineal was also use as red pigment for miniaturist and watercolour painters. The cochineal was brought to Europe in the 1512 from Spanish conquerors which learn the secrets of this plant from the natives of Mexico and import it in Spain. The name cochineal had Spanish origin since derives from the similarity with the woodworm that in Spanish was called "cochinilla".

In the century from the mid-1700s to the mid-1800s, it is estimated that 27,000 tons of cochineal were exported from Mexico to Europe, making this the second most important trade item next to silver. The high demand for American cochineal in Europe was due to the fact that the content of active coloring matter was higher than in the scale insect dyes. After

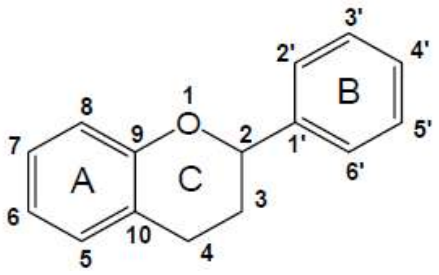
several unsuccessful attempts, farmers finally succeeded in the 1830 century to breed the cochineal in Europe, particularly on the Canary Islands, which from then on became one of the main suppliers for this dyestuff in Europe. Two grade of cochineal were avalaibles on the European market: a wild variety, called grana silvestra" and a "cultivated" one called grana fina or mestica.

To extract the dyestuff the insects were collected and killed with hot steam or vinegar before to be dry in the sun. According to an alternative method the insect could be killed and dry in a unique operation by heating in a oven on an hot iron plate.

The main constituent of all cochineal dyes is carminic acid, an hydroxyanthraquinone with a lateral chain of C-glycosyl and only one position free on the aromatic nucleus. The various species have, however, characteristic fingerprints of other minor anthraquinone components (including kermesic and flavokermesic acid), as well as of some still unidentified anthraquinoid compounds commonly named dcII, dcIV and dcVI which allows one to distinguish them in historical samples.

Fig. 2.12 - structural ***formulas of the main aglycons in cochineal extract***

2.5.2 FLAVONOIDS



The flavonoids are poliphenolic compounds derived from the 15-carbon-based flavonoid backbone. This basic structure is composed by three circle generally called A,B and C and specifically by an hexagonal heterocyclic ring (C) to which are attached two aromatic rings (A,B). The A ring is condensed to C while the B ring is linked to the C in position 2. Within this common structure the degree of oxidation and the chemical nature of the substituent on the three rings differentiate the different compounds (more specifically the degree of oxidation and substitution pattern of the phenyl ring (C-ring) identifies the different classes of flavonoids).

More than 8000 different flavonoids have been isolated from a wide range of vascular plants. They acts as secondary metabolites of plants that means that are compounds that not directly participate to the main vital functions of plants (reproduction and growing) but take part to secondary ecological ones such as broad spectrum defence (e.g. predators repellents, light screening, antimicrobials, antioxidants) and supporting to the reproduction processes (e.g. visual attractors). These are the functions of the flavonoic dyes which intensely colour plant, flowers and fruits. Their name is strictly related to their colour; the term "flavonoid" derives in fact from the Latin word flavus that means yellow. This is the colour that the most of them have in nature even though, when used as dyestuffs, can manifest different tones on the base of the extracting and using condition (e.g. pH, mordant).

A particularly class of intensely coloured flavonoids is that of anthocyanins (from the greek words *anthos* and *kyanos* which means respectively flower and blue) that are the molecules giving the red to blue colour to most flowers, fruit, roots and leaves (see the basic structure in Fig. 2.13). In nature Flavonoids mainly occur in form of glycosides, chemical compounds with a sugar group called glycone (such as rhamnose, glucose or galactose, etc) and a non-sugar group called aglycone or genin, produced in the reaction between a hemiacetal and an alcohol. All the flavonoid dyes are water soluble and belong to the technical group of the mordent dye.

Despite their complex chemistry the flavonoids have been classified according to their chemical structure into several groups as showed in figure 12.:

Fig. 2.13 - basic structures of flavonoids

BUCKTHORN

Other names: stil de grain, jaune d'Avignon, sap green, giallo di spincervino, prugna meroli, cerval, giallo santo, schüttgelb, shitgeel, Turkey berries, Persian berries, yellow berries, verde vessie, pinke

It is a dye obtained from the berries of several species of the genus Rhamnus which produce yellow and green flavonoic dyestuffs. The most common species throughout Europe is the Rhamnus catharticus L. Other minor sources of the dyestuff are the Rhamnus alaternus L. as well as the Rhamnus saxatilis Jacq. and the two sub-species Rhamnus infectorius and Rhamnus tinctorius. The popularity of this lake pigment and its wide geographical production is testified to the numerous terms used in the centuries to refer to it, many of which relate to the place of origin.

Fig. 2.14 Rhamnus catharticus L (a) Rhamnus alaternus L (b) Rhamnus saxatilis (c)

The dyestuff obtaned from rhamnus was known and used since the medieval age (starting from XIX century) for the dyeing of textiles and as pigment for miniatures. For using in illuminated manuscripts the colour was preserved in liquid syrup like form in bladder sacks. From this habit derive the common name of verde vessie (bladder green). This transparent

lake was particularly appreciated and widely used by Dutch and Flemish masters of 17th century as Rembrandt, Vermeer and Rubens.

One of the most typical recipes recommends squeezing the ripe berries and exposing the pulp to the sun in order to macerate and easily separate the juice. The extracted dye solution was later let absorbed on alum substrate. Exist a lot of different recipes to obtain a lake from buckthorn which vary in colour depending on rate of ripening and preparation process. In general to obtain a yellow dye the berries should be unripe (yellow-green coloured) and for a green one they should be well ripe (black coloured and dark). Different hues can be obtained using different mordanting agents (alum, sodium, tin, copper or iron salts) or modifying the extraction temperature (at about 50°C is produced a lemon yellow lake while increasing the temperature till about 100° C is obtained a darker, orange-colored one.

The berries contained mainly glycosylated flavonols, and in particular, rhamnosides. The aglycone parts were typically flavonol derivatives with a hydroxyl group on position 3, such as quercetin. Principal components are rhamnetin, rhamnocathartin, rhamnotannic acid and rhamnin. Minor components include isorhamnetin, chrysophanol, kaempherol, quercetin, xanthorhamin, emodin, frangulin, rhamnazin, rhamnocitrin, although this content can significantly differ.

Fig. 2.15 structural formulas of the main aglycons in Buckthorn extract

WELD LAKE

Other names: erba guada, arzica, gualda, ancorca, laque de gaude.

The weld is one of the most famous historical yellow dyes and is mainly obtained from a plant belonging to the family of Resedaceae: the Reseda Luteola L. It is a plant native of Eurasia which grows wild in most of Europe and in parts of North Africa and in north America as introduced specie. It is an annual or biennial herb which can reach the 150 cm in height. The leaves of the plant are formed in the first year and the plant evolution is complete in the second year.

Fig. 2.16 - Reseda Luteola L

Even though the dyestuffs are present in higher concentration in the greenish-yellow flowers, the whole plant was used for dyeing textiles (wool and silk) since even leaves and fruits contain colouring matters. Weld produces bright and fast yellow colours with alum or cream of tartar as a mordant. It has been the most used yellow textile dye until the discovery of America in 1492. After this date has been flanked and partly replace by the Old fustic. Its arrival in Western Europe is quite uncertain. There is a diatribe between who assert the weld has been introduced by Romans and who instead sustain that were the Moors from North Africa via Spain. It was introduced in England in the Middle Ages increasing the yellow palette of local dyers since the only yellow dyestuff employed was up to that time the dyer's broom (Genista tinctoria L.). All over the Europe Weld was soon traded as "the best yellow dye for silk and wool).

The lake was known since the ancient times (Vitruvius and Heraclius talking about it in theirs treatises) as a pure and durable colouring matter that can be as brilliant as orpiment. The alum precipitated lake was widely used in illuminations, often mixed with marble powder. It has been the most universally used yellow dye in Europe prior to the availability of quercitron at the end of XVIII century. Many recipes are known for the preparation of the pigment. One of the principals suggest to cover dried plant in a weak alum solution and then precipitate the lake by adding calcium sulfate, eggshell or lead white. The flavonoid content in weld is up to 2% by weight. The principal ones are luteolin (2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromenone) and apigenin (5 ,7-Dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) mostly occurring as their sugar derivatives.

Fig. 2.17 structural formulas of the main aglycons in weld extract

IRIS GREEN

Other names: verde giglio, lily green, viride de lilliis azurinis, gigli azzurri

It is a green lake obtained from the flowers of Iris germanica or Iris florentina. It was used above all in mediaeval manuscripts between the XIV e XV century to realize illuminations and in the watercolour paintings.

Fig. 2.18 - Iris germanica L (a) Iris florentina L (b)

In spite of its poor coating power, the pigment was particularly appreciated for its bright colour. It has been described in a lot of ancient treatise. Heraclius for example suggested to use the iris green (*suecus gladioli*) to create shadow on the verdigris. The dyestuff was extracted from the petals juice. It was generally prepared by cold maceration of the petals in a solution of gummed water and alum. The solution was then filtered and let dry. In addition to the preparation of lakes was a common practise gathers this dyestuff into clothlets (pezzette in Italian) by soaking pieces of linen into the juice of the plants and then drying and preserving them to be used when necessary. The chemical composition includes both isoflavons and anthocyanidins. The principal colouring matter is the xanthone mangiferin (2-β-D-glucosidyl-1,3,6,7-tetrahydroxy- 9H-xanthen-9-one), others are irigenin, malvidin, negretein, petanin, petunidin.

BRAZILWOOD

Other names: verzino, braza, Brasilium, brexillium, brixillium, brasileum, brasilium, lignum braxillii, verxillium, presilg, prisilje, presilje, persilje, rose lake, bressil or brezel, bracili or brazili

The brazilwood is a dyestuff derived from a number of closely related species of hard red wood belonging to Caesalpinia and Haematoxylum genus. The main ones are Caesalpinia echinata Lam., Caesalpinia brasiliensis L. and Haematoxylum brasiletto Karsten but there are also Caesalpinia japonica, Caesalpinia sappan L. and Caesalpinia violacea.

Fig. 2.19 - Caesalpinia echinata (a) Caesalpinia brasiliensis (b) Haematoxylum brasiletto (c)

The wood was imported into Europe since medieval times. In that Age the known and employed brazilwood was extracted from C. braziliensis coming from Middle East. In the 1120 a new source of the dye was introduced in Europe at the hands of the Portuguese. It was the Southeast Asian tree Sappanwood (C. sappan L.).The dye had a beautiful red colour that reminded to that of burning coals. Several authors suggest that the ancient name brazil come from the Portuguese word "brasa" which means ember. Starting from the XII century the Brasil is mentioned in a number of Italian dyeing treatises. It was use as mordant textile dye (with tannins and alum to dye cotton and with alum or cream of tartar to dye wool) often in mixture with other more precious dyes (such as madder or kermes) and for the preparation of lake mainly destined to illuminations.

But the really revolution begins with the discovery of America, when Portuguese explorers found that the new lands were rich of trees very similar to the brazilwood ones. They baptized the tree *Palo de brazil* and the land when they grew Brazil. As a matter of fact this dye was well known to the pre-Columbian civilization. It was for exampled known to the Aztec with the name *nacazcolotl* or *vitzquavitl*[2].

[2] Florentine Codex' (Florence, Biblioteca Laurenziana MS Palatina 218–220)

The Cesalpinia echinata, which was so abundant at the time of arrival of the Portuguese is now included in the IUCN red list of threatened species and can be found just in botanical gardens and national parks.
The principal compound contributing to the reddish colour of Brazilwoods is the brazilin (3,4,5,7-tetrahydroxy-2,3-methelen-neoflavan) which through autoxidation develops into the red dye brazilein. With the aging the dye develops another yellow compound denoted "type C" which is not already chemically characterized.

Fig. 2.20 - Structural formulas of the main aglycons in brazilwood extract

CAMPECHE

Other names: logwood, blockwood, campeachy, peachwood, haemasol, hematine, Jamaica logwood, Indishout, kampech hout, legno campeggio, pecho de Campeche, provintiehout, blauwhout, blutholz

Is a red-violet dye derived from the bark of tree of the genus Haematoxylum, principally from Haemotoxylum campechianum L. and Haemotoxylum brasiletto Karst. These plants are natives of southern Mexico (area around Campeche bay, in Yucatan Peninsula) and northern Central America, and were introduced in Europe in sixteenth century by the Spanish explorers.

Fig. 2.21 - Haematoxylum campechianum

In the 17th and 18th centuries the dyestuff was often used in combination with other dyes (as brazilwood) to give the fabrics a basic colour. It was transported to Europe it in the form of large blocks, hence the names logwood and blockwood.
The main colorant substance present in logwood, the haematoxylin, is quite similar to the brazilin. In effect is the same molecular structure with a oxydrilic group more. About 10% of the duramen is composed of hematoxylin, with tannins and resins also present. During the dyeing process the hematoxylin oxidises to the strong chromophore haematin, a red dye which can assume different colour (red, purple, blue, black) depending on the mordant used and on the pH. To preparate the dyestuff the wood was shredded into chips which were put to ferment with a 30% water in order to obtain the aglycon hematoxylin and oxidate it to hematein. The process could last up to 6 weeks but coyuld be acelletated by addition of ammonia (traditionally in the form of urine).

Fig. 2.22 structural formulas of the main aglycons in campeche extract

SAFFLOWER

Other names: Saffron; Assiette rouge; Pink saucers; Rouge; Rouge végétale; Saucer colour, cartamo

Safflower is the common name of Carthamus tinctorius L., an annual herb belonging to the Compositae family used since antiquity to provide orange, red and yellow dyes. The name Safflower is derived from "saffron" because of the chromatic similarity between the two dyestuffs. The word carthamus instead seems to be derived from the Arabic name of the dye *quartum* or *gurtum*. The specie is indigenous of southern Asia but it has been early cultivated in the entire Mediterranean basin as well in southern Europe and China.
The dyestuff was obtained from the petals of the orange flowers. The flowers were usually collected during the morning shade, dried on muslin trays and then stored in tins. Early users had already developed an extraction method which permitted to isolate in a first step the yellow dye and in a second phase the red one. Normally the yellow dye components were separated by repeatedly soaking the flowers in coldwater until all the yellow matters was completely removed.

Fig. 2.23 - Carthamus tinctorius L

Then the red dye was extracted by immersion in an alkaline solution (sodium or potassium carbonate). Since the red one is a direct dye the filtered solution direct constituted the dying bath were the textiles were immersed to be coloured. The dye was precipitated into the fibres by adding an acid solution (such as vinegar or lemon juice) as neutralizing agent. The yellow dyestuff collected in the first extraction was used to dye mordanted wool and silk. Furthermore the both were used for the production of painting pigments.
The literature sources testify the early employ of the dyestuff. The first documented finding dates back to 4000 years ago in an Egyptian tomb. Most recent but very interesting has been the finding of a bunch of Carthamus flower wrapped in willow leaves, buried with a mummy dated to 1600 BC. In the medieval time had a great diffusion and fortune IN Europe and it was profitably cultivated in Italy, France and Spain. Then, after the discovery of America, the Spanish exported it to new word by realizing crops in Mexico, Venezuaela and Colombia.
The principal use was as fabric dyestuff but there are a number of records of its conversion in pigments for various purposes. In Orient it was also use to prepare herbal medicines and cosmetics; in Japan is quite famous its employ to create cosmetics for geisha and kabuti artists.
The colouring matter is composed by a red insoluble fraction and a yellow soluble one. The principal red colour constituent is the carthamin (C.I. Natural Red 26) a quinochalcone

produced in the petals during la late blooming period from the yellow precursor precarthamin. It is composed of two chalconoids whose conjugated bonds cause the colouration. Other minor red dyes are isocarthamin and isocarthamidin.
In the petals are also presents 6-hydroxy-kaempferols, hydroxysafflor yellow A, safflor yellow A, safflor yellow B, safflomin A, safflomin C, isosafflomin C, tinctormin, precarthamin, anhydrosafflor yellow B, and cartormin. These compounds, all belonging to the family of flavonoids and accomunated by the same C-glycosylated quinochalcone moiety, constitute the yellow water soluble colouring fraction. As happen for many natural dyes the chemical composition is subject to significant variations depending on cultivar.

Fig. 2.24 - Structural formulas of the main dyestuffs in safflower extract

OLD FUSTIC

Other names: legno giallo, dyer's mulberry, yellowwood, fustic, bois jaune, murier de teinturiers, gaud, Madera amarilla, moral fustete, geelhout, aurantica, calicogelb, carmin de Cuba, fustetto vecchio, gelbholz

Old fustic, also called dyer's mulberry, was one of the most important yellow dyes in Europe from the 16th century to the early 20th century. It is obtained from the heartwood of the Chlorophora tinctoria L. tree (or Maclura tinctoria), a moraceous specie indigenous of central America. The European found the plant in the American tropical forests after the 1492 Columbus' discovery of the continent. Shortly they notice that the wood of old fustic trees made a good yellow dye. Due to his relation with Mulberries, is the scientific old name morus tinctoria L. that is sometimes still use to indicate the tree (*morus* come from the genus of mulberries and *tinctoria* to indicate that it is a dyeing plant).

In a very short time after its arrival in Europe, the old fustic was adored by the dyers, and, thanks to its great stability, became the yellow dye par excellence. It was in fact a very good colouring agent which provided brilliant and strong colours and a superior colourfastness than the weld. Hundreds of thousands of tons of old fustic were imported to Europe from America during the last 500 years. A curiosity related to the history of Old fustic is that it was the dyestuff employed to produce the khaki fabric for the U.S army uniforms during First World War. In artistic sector it has had a limited use as a watercolour. Its employ in the early nineteenth century for the manufacture of fine yellow lakes is also chronicled.
It do not be confounded with the Young fustic, a yellow pigment obtained from the wood of Rhus cotinus a shrub or small tree native of southern Europe. The young fustic, also called sumac, was widely use in the medieval Europe but not had a great fortune (in spite of the great availability of this plant) because of its very poor colourfastness.

Moreover it was cheaper (the weld was a crop and necessitated a lot of work to be obtained) at the time very abundant. Since the young fustic was earlier known, presumably the Old fustic owes its name to the chromatic similarity between the two dyes. As regards the original name "fustic" apparently comes from the Arabic word for bush.

Nowadays the Old fustic continue to have a discrete importance unlike the majority of the other naturals dyes that are be almost completely substituted by synthetics ones. It is especially used in tanning industries to produce particular shades of leather.
The main colouring agents of old fustic are the favonols yellow dyes morin (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) and kaempferol (3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) and the benzophenon maclurin ((3,4-dihydroxyphenyl)-(2,4,6-trihydroxyphenyl)methanone).

Fig. 2.27 - Structural formulas of the main dyestuffs in old fustic extract

2.5.3 INDIGOID DYES

The name indigoid derive from the forefather of this dye class, the indigo, a blue dye characterized by the chromophore illustrated in Fig 2.28, where X is a differently complex aromatic group. It is a conjugated system that can present a symmetry plane in respect to the double bond. Consequently the indigoid dyes can be symmetric or asymmetric according as the two X substituents are identical or different. In addition, since there is a double bond, they can present cis-trans isomerism.

Fig. 2.28 indigo molecule (a) and its chromophore (b)

From a technical (tinctorial) viewpoint they all belong to the category of the vat dyes. Indigoid dyes can give a varied palette ranging from yellow to black but the natural ones tend to assume bluish-purple tones. All the dyes belonging to this group are indifferently characterized by a high light fastness and a good resistance to acid-basic attack, but present a very poor stability to the action of oxidizing and reducing agents. Other important common feature of indigoids is that they are not present in nature as dyes but as dyes precursors.

INDIGO

Other names: anil, azorium Romanum, Baghdad indigo, Inde blew, indebaudias, indich, indicum, Indigo Bagadel, lamptschen endich, Lombardy indigo, lulax; pastel

Indigo is a blue dye that can be extracted from species of many different plants found in several parts of the world. These include several species of the Indigofera genus, the Isatis tinctoria L. and the Polygonum tinctorium (dyer's knotweed or Chinese indigo native to eastern Europe and Asia). The indigo obtained from Isatis tinctoria have given a such important role in history to gain itself the consideration of independent dye (see woad). The most important source of indigo is undoubtedly the Indigofera tinctoria a papilionaceous (Leguminosae family) plant indigenous to India, South and Central America, Asia. The dye precursor, called indican, is contained in leaves and stems.

Fig. 2.29 Indigofera tinctoria L (a) Polygonum tinctorium L (b)

The dyes was known to the ancient Mediterranean civilization; Vitruvium talk about it in his architecture treatise in the 13 B.C. The name indigo derives from the greek ινδικον and the Latin indicum, meaning "coming from India". It was used by Greeks and Romans principally as a painting pigment, not as a textile dye, for which they preferred the native woad. Plinyfor examples describes its use as a fresco pigment. In India was instead used as dyeing colorant since the third millennium B.C. Extensive research had demonstrate that indigo was employed (both with palygorskite and attapulgite) for the preparation of Maya blue, the wonderful bright blue pigment used by the Mayans for painting on murals, sculptures, ceramics and textiles. Several mediaeval manuscripts reports information about the use of imported oriental indigo as a pigment; the fifteenth century Bolognese manuscript, gives nine methods for the preparation of indigo for use it as a pigment. The first description of the Indian efficient indigo preparation method come to Europe thanks to Marco Polo (1254-1324) who describe it in his travel diaries.

From that moment, starts the fortunate trade of indigo mainly taken over by the Genoese and the Venetians merchants. The indigo was mentioned in all merchants' account books and in customs tariffs. The success was so great that by the mid-seventeenth century, the Indian indigo had almost completely replaced the European source of indigo, the woad. As a result of huge demand, indigo was cultivated on plantations, often in colonial lands. The indigo commerce has been a lucky business for a lot of men; one of them, the famous archaeologist Heinrich Schliemann, went down in history for the discovery of the ancient Troy, make possible thanks to the proceeds of the indigo commerce that financed the excavations. The fortune of indigo cultivation ends in XIV century consequently to the introduction of the synthetic dye discovered by Baeyer and Drewson in 1882.

The pigment was generally considered too dark for direct use and was therefore mixed with various white pigments (e.g. white clay, calcium and magnesium carbonate) and others inorganic materials (aluminium and iron oxide).

The dye was extracted from the fresh leaves in water after about 9 hour in maceration in an alkaline solution. The adding of alkali in important because it facilitate the starting of fermentation (some processes also implicate the addition of urine, ash or slaked lime). The fermented leaves mixture must be frequently and vigorously stirred in order to oxidise the

leuco-indigo and develop the blue dye. The obtained pulp was later heated in order to block the fermentation process and then gathered and pressed into cakes.
Since indigo is a vat dye, to use it as dying agent, it is necessary reduce it in a intermediate soluble leuco form that may bond with the fibers. To develop the color the texiles must be later expose to the air to be oxidated from the atmosferic oxigen. This reaction rapidly oxidizes the leucoindigo and produces the blue, water insoluble indigo.

Fig. 2.30 reversible reduction to leuco-indigo and irreversible oxidation to dehydroindigo (yellow)

The main colouring agent detectable in indigo are the indigotin (2,2'-Bis(2,3-dihydro-3-oxoindolyliden)) and indirubin (3-(1,3-Dihydro-3-oxo-2H-indol-2-yliden)- 1,3-dihydro-2H-indol-2-on). Since indirubin is a red dye, it gives to indigo a purple hue. In tropical and subtropical climate, indigofera species also contain indican. The optical properties of indigo are due to the cross conjugated system of donor and acceptor groups linked by a C=C double bond. The potentially acidic protons are held in close proximity to the potentially basic carbonyl oxygen atoms by means of hydrogen bonding in the ground state.

Fig. 2.31 structural formulas of the main dyestuffs of indigo extract

WOAD

Other names: Isatis, vitrum. Glastum, weit, weild,guato, guado, pastel

The term woad may refers to both to the plant and the dyestuff derived from the plant Isatis tinctoria L. native to the Mediterranean and Western Asia, and Isatis indigota from China. It is one of the earliest known sources of indigo worldwide.

Fig. 2.32 Isatis tinctoria L.

Isatis tinctoria, a biennial species of the Family Cruciferae (or Brassicaceae), had been historically cultivated in Europe from the 12th to the 17th and was the centre of a large industry based on the production, extraction and dyeing with indigo. The mains production centres were in England (Somerset, Lincolnshire), France (Normandy, Somme, Languedoc), Germany (Jülich, Thuringia) and Italy (Piedmont, Tuscany).

The use of woad is reported in a number of ancient sources. Pliny for example describe the habit diffuse among the ancient Britons to use the dye for colouring their body to scare the enemies. The fortune of the woad industry begun to decline when Marco Polo introduce from Orient the receipt to dye with the Indian indigo which was more efficient and provide a more brilliant and pure colour. Woad cultures disappeared completely with the advent of synthetic indigo in the late 1890s.

The dye precursors in woad are the isatan B (indoxyl-5-ketogluconate), the main one with a percentage of aprox. 80%, and the indican. In the process the sugar element is cleaved trough hydrolysis from the indoxyl group and two of the resulting indoxyls combine to produce the indigo molecule. If the precursors are oxidized before the indigo is formed, indirubin is also formed (see Fig. XXX). This chemical reaction starts spontaneously when the plant tissue is damaged and expose to air (so could begin immediately after the harvesting).

The traditional method for the production of indigo exploits this process occurring directly in the woad leaves. The leaves were harvested and immediately crushed in a specially designed mill in order to obtain a pulpy paste. This paste was used to create woad balls measuring bout 10-12 cm in diameter which were let to dry (and oxidize) for about 4-6 weeks. Then the balls were crushed until reduce the woad to a powder which was charged with water and urine, and let ferment aerobically during some weeks. At the end of the process the blue pigment was ready to be used by dyers or artists. This production

technique would be unacceptable nowadays as it is inefficient, malodorous and produce a dirty indigo. As suggest in modern studies the process could be improved by extraction of the soluble precursor in anaerobic condition (to avoid the preliminary oxidation) separating it from the leaf tissues, and subsequently convert the precursors in indigo.

Fig. 2.33 indigo formation from the precursors isatan B and indican

TYRIAN PURPLE

Other names**:** Purple, purple of the ancients, royal purple, Tyrian purple, shellfish purple, Byzantium purple, Ostrum, porpora, purpurissum

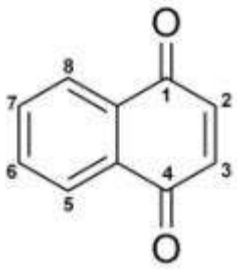
The purple is one of the oldest and most famous dyes of the history. The coloring matter is extracted from gastropod mollusks of the Muricidae family. The most common ones in the Mediterranean area were the Murex brandaris (Bolinus brandaris L.) also known as spiny dyemurex, the Stramonita haemastoma (Thais haemastoma L.) known as red-mouthed rock shell and the Murex trunculus (Hexaplus trunculus L.) also called banded dyemurex.

Fig. 2.34 Murex brandaris (a), Stramonita haemastoma (b), Murex trunculus (c)

The purple was mainly used to dye precious textiles and parchments since it was considered a luxury good. Were required approximately 12.000 shellfish to extract only two grams of dye sufficient to dye about 2 Kg of wool). The word purple is derived from the Latin word "purpura" the Greek word "porphyra". The dye is mentioned in a number of ancient source. The first recorded reference to the dye was found in Crete and date back to 1600 BC.
Is a well-known fact that the Phoenicians were the leaders in the production and employ of purple dye (not by chance the most famous is the Tyrian purple) and that they used to falsify this dye superimposing a thin layer of diluted purple to other less valuable ones (e.g. kermes, blueberry, mauve or orchil dyes). The dye has been considered since the origin a status symbol. In ancient Asia was reserved to the rulers habit then adopted by Alexander the Great in Macedonia; In the early Roman culture only chiefly members of the priesthood could wear the purple dyed pallium. With the Republic this privilege was reserved to high officials of the state (purpurati) while under Caesar and Augustus was a symbol of to distinguished specific office and rank. In the following centuries continued to be used by highest dignitaries of empires and religious institutions. To obtain the precious pigment the mollusks were collected, triturated and them let dry in the sun during about three days. Then they were boiled a long into lead pots. The dyestuff precursor, a viscous colorless substance, is contained in the hypobranchial glands of the mollusks. This secretion in mainly constituted of sulfate esters of indoxyl, 6-bromoindoxyl and derivatives of these compounds with methyltio or methyl sulfonyl groups in the position 2. When this secretion is exposed to the air, they are first converted in a greenish substance by means of an enzymatic hydrolysis and then, trough a photochemical conversion, to the purple pigment.

The fascination and interest always aroused by molluscan purple has resulted in a deeper study of the dye, involving both historical and technological aspects of its production and chemical composition. Actually, a wide number of articles and reviews exploring the chemistry of this dye is available in the scientific literature. Its composition includes about 10 brominated and un-brominated colorants comprising indigoids, indirubinoids and isatinoids. The dye could have different hues depending on the specific specie used and to the dyeing technology. Recent studies conducted on various species of muricidae had in fact shown that Murex brandaris and Stramonita haemastoma contained as precursors only 2-substituted 6-bromoindoxyl, while Murex trunculus contain also indoxyls and 6-bromoindoxyl unsubstituded. This have as consequence the obtaining, by using this third, of a bluish color due to the presence of both 6,6'dibromoindigotin and indigotin. Starting from the identification of components and of their respective ratios, a method for identifying the dye source is therefore proposed. According to the authors Murex trunculus is the only molluskan species producing significant amounts of 6-bromo indigotin. (Koren, 2006-2013)

2.5.4 NAPHTHOQUINONES



The naphthoquinones are a group of natural and synthetic dyestuffs derived from naphthalene and based on the naphthoquinone skeleton (see figure) also class of natural phenolic compounds formed on a C6-C4 skeleton. As well as the anthraquinones they belong to the macro-class of quinonic dyes characterized by the presence of a quinonic group as chromophore. In nature they can be found in leaves, blossoms, wood, bark, roots and fruit even though to a lesser extent compared to te parents anthraquinones. Several of them, as for examples the alkannin and alkannan, have been found employed in the formation of lakes and other pigments. They were used as direct, acid or mordant dyes producing coloration ranging from pink to black passing through orange, red and brown hues.

ALKANNA

Other names: dyers' bugloss, orchanet, alkanna, orcanette, dyer's alkanet, anchusa, orkanette, palomillo de tintores, orcaneta, orosma echioindes, schminkwurzel

Alkanet (Alkanna tinctoria) is a Mediterranean plant belonging to the boraginaceous family, whose roots have been historically used to produce a reddish dye. A second accepted source for the alkanet dye is the plant Anchusa tinctoria that is member of the same botanical family. Exists two other boraginaceous plants related to the alkanet (Pentaglottis sempervirens L., also known as alkanet, and the Lithospermum arvense L., known as bastard alkanet) but their use as dying agent is so far uncertain. The alkanet plants have little bright blue flowers and reddish purple roots that once powdered are used to extract the dying matter.

Fig. 2.36 Alkanna tinctoria (a), Anchusa tinctoria (b)

The first record of use of the alkanet dates back to 4th and 5th centuries BC (it was contemplated as medical plant for the treatment of ulcers in the writings of the Greek doctor and philosopher Hippocrates) however its use as fabric dye is almost certainly more ancient. Is was mentioned by both Pliny and Theophrastus, who talked of the blood red roots of alkanet as a dyestuff. According to ancient sources it was used to a certain extent in the late Egyptian Age in Egypt and in neo Babylonian times in Mesopotamia. Seems that in the Medieval time the alkanet fell in disuse as fabric dyestuff but continue to be used for the realization of cosmetics, calico prints and pigment until the beginning of 19th century.
The main chemical compounds that constitute the dye are alkannin, alkannan and shikonin. Alkannin (5,8-Dihydroxy-2-[(1*S*)-1-hydroxy-4-methylpent-3-en-1-yl]naphthalene-1,4-dione) is a naphthoquinone soluble in organic solvents and very sensible to pH changes (the colour varies from red at pH 6.1, to purple at pH 8.8 and blue at pH 10.0).

Fig. 2.37 **- structural formulas of the main dyestuffs safflower extract**

2.5.5 CAROTENOIDS

Carotenoid are tetraterpenoids molecules, which means that they are constituted by 8 isoprene units containing 40 carbon atoms. They can be considered derived from the acyclic $C_{40}H_{56}$ structure shown in fig. xxx, having a long central chain of conjugated double bonds.

According to their structure these molecules general absorb blue light and are consequently yellow-orange coloured. They are very common in nature (nowadays there are over 600 known natural carotenoids) and are responsible of important biochemical functions in plants and algae: absorbing light energy for use in photosynthesis and protecting chlorophyll from photo-damage.

SAFFRON

Other names: safran, zafferano, echter safran, azafrán, safraan, fan-hung-hua, safran, saufuran,

Saffron is obtained from the red stigmas (pistil apical part) of the flowers of Crocus sativus L. a plant belonging to the Iridaceae family native to Greece (or to Southwest Asia).
Since ancient it had being used as a spice for flavoring and coloring food preparations, as a cosmetic (for giving color to skin, hair, nails and lips), a texile dye or coloring agent. Its medicinal use has been also reported. Nowadays the plant is widely spread and cultivated in all the world but its use is limited to the gastronomy sector.

Fig. 2.38 – Crocus sativus

The ancient Persians and Babylonians used the saffron to dye shoes, the Egyptian to color mummies bends while the Romans to dye the wedding veils. In the Roman Empire the dying with saffron was so important that the dyers who used saffron were known as crocotarli. With the pass of time it was replaced by cheaper dyes as the weld.

As fabric dying it could be used as direct dye or as mordant one. While as direct dye produce a bright orange-yellow color, in the other case produce a opaque yellow with tin and an opaque orange with alum. The textile was boiled in the coloring bath.
As pigment, saffron was used mainly by illuminators due to its high cost and its poor stability to the light. In this particular artistic sector it had a considerable importance as demonstrate the numerous citations and recipes in documentary sources. Worth to be mentioned its use for simulate gold leaf, usually by superposing a thin layer of saffron to an orpiment one.

The substances contained in the saffron can be categorized as follow: dyes, compounds responsible for the peculiar bitter taste (e.g. picrocrocin) and aromatic compounds responsible for the distinctive odor and aroma (e.g. safranal). In addition, saffron contains proteins, sugars, carotenes-vitamins, flavonoids, amino acids, mineral matter, gums and other chemical compounds. The main colour-imparting constituent is crocin, a digentiobiosyl derivative of crocetin (8,8'-diapocarotenedioic acid), an apocarotenoid quite unusual as it is water soluble in contrast with most carotenoids found in nature

2.5.6 TANNINS

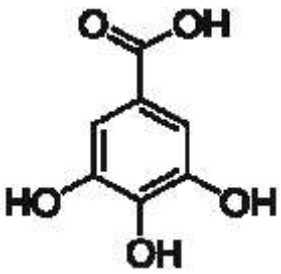


Fig.2.39 - Gallic acid

Tannins are polyphenolic compounds very common in vegetal world. They are characterized by high molecular weight (ranging from 500 to 20.000), solubility in water (with the exception of some high molecular weight structures) and ability to form with proteins insoluble or soluble complexes. Tannins have been largely used in history in the leather industries for the tanning process and in the artistic world for the production of pigments and inks such as the famous Iron gall ink. They are divided into two groups, hydrolysable tannins and proanthocyanidins, depending on their structure. Hydrolysable tannins have a carbohydrate (generally D-glucose) as central core, a 6 to 9 galloyl units skeleton, and presents partial or total esterification of hydroxyl groups by means of phenol molecules. On the base of the phenol involved in the process we can classify the hydrolysable tannins into two main groups: gallotannins (gallic acid), ellagitannins (ellagic acid). Their name derive from their tendency to easily be hydrolysed (forming carbohydrate and phenolic acids) by mild acids and bases, hot water and enzymes action.

Proanthocyanidins are much more widely spreader in nature than hydrolysable tannins. The name derives from their capacity to produce anthocyanidins via acid catalysed oxidation reaction. They are oligomers or polymers of flavonoid units (2 to 50) linked by carbon-carbon bonds. Unlike the hydrolysable tannins they are not susceptible to cleavage by hydrolysis. They are also known as condensed tannins due to their condensed chemical structure. As the flavonoid units can present different substituents and the position on the interflavan bonds can vary in the molecules, proanthocyanidins are usually characterized by complex structures. They can be water soluble or insoluble depending on their chemical structure. Nowadays are preferentially included into the flavonoids class.

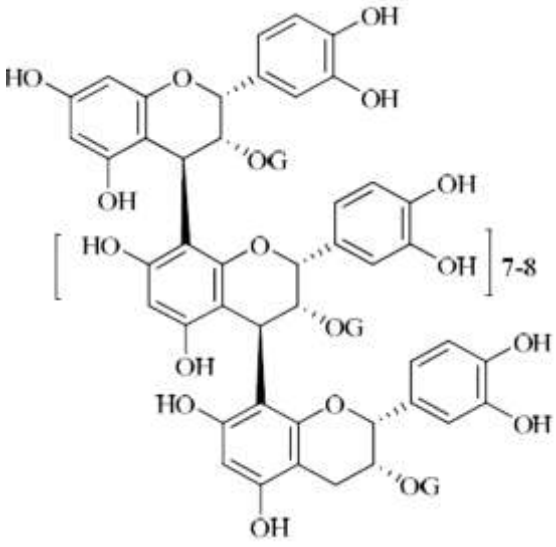
RATANIA

Other names: rhatany, rattan, rathania Peru.

Ratania is a dye obtained from the dried root of the species Krameria triandra and to a lesser extend Krameria ixina and other undetermined species of Krameria. The name Ratania, which identify both the dye and the plant, literally means "the plant creeping on the floor". The plant is in fact native to Bolivian and Peruvians Andes where it grows in very hard conditions on sterile sandy slopes of the rocky mountains. This is possible thanks to the powerful roots, rich of branches and very strong. In the roots is accumulated the reddish brown dye.

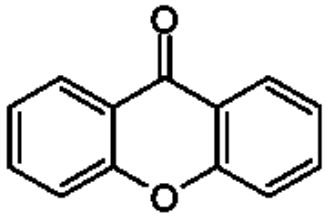
Fig. 2.39 - Krameria triandra

The root extract has been used as a textile mordant dye giving colours varying from brownish-red to flesh tone. With ferric salt were obtained dark green hue. As well as to produce the dyestuffs the ratania has been largely used in natural medicine for antibacterial, astringent antinflammatory and antioxidant purposes. The roots have historically been used by the natives of Bolivia and Peru to care for their teeth. The roots are characterized by an high contents in tannins localized mainly in the cortex. A number of oligomeric proanthocyanidins are formed by a variable number (2-14) of propelargonidina and procyanidins. They are almost colourless but tend to accumulate and condense forming insoluble compounds called pink phlobaphenes. This macromolecules are the principal responsible of the Rathania red. Even if the the global chemical composition is very complex (and not at all known yet) to simplify it could be said that Kramero-tannic acid (or ratanhia-tanic acid), rhatannin, and rhatanic red are the principals compounds constituting the dye.



rhatannin

2.5.7 XANTHONOIDS



Xanthonoids are natural phenolic compounds which structures are based on the xanthone backbone. This structure, having three fused hexagonal rings, is quite similar to that of anthaquinones with the difference that one of the central carbonyl group is substituted by an oxygen atom.
Xanthonoid compound have been used to produce a variety of pigments apparently really dissimilar. Among them the most famous include indian yellow (euxanthone and euxanthic acid), dragon's blood (dracorubin and dracorhodin) and gamboges.

GAMBOGE

Other name gommagutta, gambogium, gumboge, gum-booge, gumbouch, gummi gambogia, gummi gutti, gamma gitta, gom guttae

Gamboge is a yellow substance of vegetable origin produced by various species of the evergreen trees of the family Guttiferae indigenous of South East Asia. The principal one is the Garcinia hanburyi which grow above all in Cambodia and Thailand. Not surpriseling the name derive from Cambodia, via the ancient name cambodiam followed by the intermediate versions camboja and camboge. Other remarkable species are G. morella, G. cambogia (from India and Sri Lanka), G. elliptica and G. heterandra (from Myanmar).

Fig. 2.40 - Garcinia species

The Gamboge present a significant yellow resin portion (70–80%), where also resides the main colouring agent, and a water-soluble polysaccaridic one (10–25%). Thus it should be correctly classified as a gum–resin. The dye, characterized by a intense golden yellow hue, come to Europe thanks to the English, who imported it in the XVII century. It was well known and widely used in China and Japan since the 8th century.
Between the XVI and the XIX has been particularly appreciated and employed by Flemish artists. It was often used in combination with blue pigments to create green ones. The XVIII century green pigment known as Hooker's green was a mixture of Gamboge and Prussian blue. Because of its chemical composition it was particularly adapt to be applicated in a simply water dispersion or with little addition of water soluble binding media such as gums or glues. For example Gamboge has been identified in the watercolour box of the

romantic painter J.M.W. Turner3. During the seventeenth century it was frequently used for tinting prints. The use of Gamboge was progressively abandoned probably due to its toxicity, its poor light fastness and to the high production costs. The resin was collected from the trees by making spiral-like cuts down the trunks and gathering the resin into bamboo canes. The gamboges naturally solidified into the bamboo and took the form of a tube that was called pipe gamboges. The tree must be at least 3 years old to be tapped.

The gamboges is characterized by a very complex chemical composition. The main resin components are the gambogic acid ($C_{38}H_{44}O_8$, also called β-guttiferin) and the α-guttiferin ($C_{29}H_{36}O_6$). Others compounds also present are gambogin, morellin dimethyl acetal, isomoreollin B, morellinol, desoxymorellinol, moreollic acid, isomorellic acid, isogambogic acid, gambogenic acid, gambogenin, isogambogenin, desoxygambogenin, gambogenin dimethyl acetal, gambogellic acid, neogambogic acid and hanburin. Many of its constituents are cytotoxins that make the pigment extremely poisonous.

[3] Townsend, J.H. 'The materials of J.M.W. Turner: Pigments' Studies in Conservation 38 (1993) 231–254

2.6. STATE OF THE ART IN THE ANALYSIS OF DYESTUFFS

Various methods have been developed for the characterization of organic dyes. In the last years the researchers focused their attention mainly on the development of new methods and techniques for analysis in situ. This is undoubtedly the way to follow for the future of Conservation Science to avoid the big problem of samples collection in precious artefacts. Nowadays, even though many advances have been done, the collection of micro samples seems to be the only possibility to obtain reliable information about a number of art materials such as dyes and organic pigments. This explain the almost complete absence of literature on the topic.
Although non-invasive techniques proved not to be decisive in the analysis of dyestuffs, in some cases useful preliminary information can be obtained from in situ surveys.

In a recent study Gulmini et al. applied Vis-FORS technique and HPLC-DAD-MS to the analysis of dyed fabrics in order to compare the effectiveness of non-invasive approach versus invasive ones. They analysed a set of wool and silk reference samples, dyed with various dyestuffs including three red dyes (cochineal, brazilwood and madder), four blue dyes (woad, indigo, logwood and Saxon blue) and four yellow dyes (weld, fustic, saffron and turmeric). Results shown that, although the technique does not give conclusive information, it is a good tool for a preliminary approach to the artifact. Furthermore, the authors identified the concentration as a very critical parameter for the direct identification of the dyestuffs since too intense and too faint colors prevent the detection of the diagnostic reflectance signals. [1]

In last 10 years Surface Enhanced Raman Spectroscopy (SERS) has proved to be a promising tool for the identification of various natural dyes. It is a Raman spectroscopy surface-sensitive technique endowed with a great sensitivity that make it particularly suitable for Cultural Heritage diagnostics. In a SERS equipment the enhancement of Raman scattering signal is obtained by adsorption of the target molecules onto rough metal surfaces or designed nanostructures.
By doing so normally weak Raman scattering is enhanced up to seven orders of magnitude and at the same time, dye fluorescence is effectively reduced. The SERS technique found a perfect applicability in dyestuffs analysis, because it acts on organic molecules characterized by large delocalized electron systems. SERS was first used for this purpose by Guineau in 1987, when he obtained SERS spectra of alizarin extracted from madder dyed textile samples. [2,3] The technique was still rough and required big samples to be applied. It had to wait about twenty years before an improvement that makes it applicable to Cultural Heritage.

Interest in this technique is demonstrated by the many publications on the topic including both studies of reference materials (providing high-quality, detailed spectra of a number of dyes) and cases studies. [2-19] To date, all the most important dyestuffs belonging to anthraquinone, flavonoid, and indigoid types have been explored with this technique.

Particularly considerable is the work carried out in The Metropolitan Museum of Art (Met) scientific laboratory, reviewed in a recent publication by Casadio et al. [4]. During their research activity they developed a micro invasive analytical protocol that successfully applied to a wide variety of art object, involving textiles, polychrome objects, works of art on paper, and paintings [4-11]. Actually the Met is today one of the reference centres for the SERS technique applied to Cultural Heritage studies. They designed and tested a lot of nanostructured surfaces and colloids in order to optimize the operative conditions for various classes of art materials. According to the authors the method allows the identification of dyes from samples as small as 25 μm in diameter.

The protocol involves the use of Silver Island Films (AgIFs), Silver Films over Nanospheres (AgFONs) and Silver colloid. The first two have been mainly applied to the analysis of red dyes (alizarin, carminic acid, and laccaic acid) while the second to investigate samples from textiles, works of art on paper, polychrome objects, and paintings, ranging in age from antiquity to the 19th century. Regarding the lakes the normal protocol involves a preliminary de-complexation treatment (hydrolysis of the dye-metal complex by exposing the sample to a HF saturated atmosphere) [6] but studies on the direct analysis of mordanted samples are being carried out. As pointed out by the authors themselves, the method is a very valuable tool in a museum context, due to the possibility of a rapid response with an ultra-micro sampling (< 50 μg), but is still far to reach the completeness of information derivable from an HPLC analysis.

In 2006 Kui Chen et al. published a study on direct identification of anthraquinonic dyes on painting fragments using SERS. The study had the declared goal to provide an easy procedure that allows to eliminating the sample preparation step. In spite of the author declared to succeed in the task, some consideration must be done. In the experimental samples is reported that the painting has been prepared by mixing the dyes with water/ethanol; no binding media have been mentioned, so that it isn't clear how the pictorial film was formed. In addition they analyzed commercial alizarin and lac dye without previous conversion in the respective lakes, eliminating in this way the factor that made necessary the sample pre-treatment [14].

Between 2008 and 2010 Jurasekova et al. published a few papers showing their successful application to the SERS technique to the analysis of dyed fibers. They succeeded in identify flavonoid and anthraquinonic dyes in historical samples without previous hydrolysis the mordant-dye complexes. The authors succeeded in identify Alizarin/Purpurin and Carminic acid in linen and wool fibers dyes with Madder and cochineal respectively, luteolin and apigenin in silk and wool fibers dyed with weld. [15,16]

In 2010 Rosi et al. proposed the Subtracted Shifted Raman Spectroscopy (SSRS) as valid alternative to the SERS technique [16]. In SSRS the fluorescence background typical of Raman spectra is reduced through a mathematical treatment of data. According to the authors the application of these methodology do not require a particular manipulation of the sample and represent a great advantage in the analysis of dyes because allows to work in resonance Raman conditions, that is in same case the unique way to enhancing the weak scatterings of the dye over the signals of the matrixes on which they are supported.

Another alternative to SERS was given by Casadio et al. whom in the same year published a study focused on the direct identification on early synthetic dyes on paper. [17] In the studies a set of illustrated broadside prints of the artist José Gaudalupe Posada have been analyzed by means of FTRS (Fourier Transform Raman Spectroscopy). The main advantage observed by the authors was the effective reduction of fluorescence thanks to the use of a Near Infra-Red (NIR) laser, which, combined with the easy and fast acquisition and manipulation of data, convert it in a suitable method for a rapid in-situ analysis of works of art. The inability to unambiguously identify similar dyestuffs, and the difficult identification in case of mixture or low concentrations, have been highlighted as important limitations.

Claro et al. presented an 2010 an interesting work exploring the potential of confocal microfluorescence spectroscopy for the analysis of painting and textiles samples. [18] They performed a set of preliminary experiments on model paints based on alizarin, purpurin and eosin red lakes (weak, medium and strong emitters respectively). The information acquired were used as reference to identify such lakes in cross sections from paintings by Lucien Pissarro and Vincent van Gogh, and in a selection of pre-Columbian Andean textiles sample. The results of a selection of samples were compared with those obtained by means of HPLC-DAD-MS reveling the limitation of the technique declared by the author not completely conclusive for the purpose.

An alternative technique suggested for the analysis of organic dyes is the Square-Wave Voltammetry (SWV) proposed as a fast method to identify both solid and dissolved samples without the necessity of any sample pre-treatment or preliminary dissolution and using a

minimal amount of sample (<1 mg) [19-21]. In these studies electrochemical measurement of several anthraquinonic, flavonoid, curcumoid and indigoid dyes, were performed using a paraffin-impregnated graphite electrode (PIGE) and the results compared to traditional methods, in particular with microchemical tests and Thin Layer Chromatography (TLC) analysis. Even though the technique was demonstrated to be suitable for the identification of several dyestuffs in textiles, it is inappropriate to complex matrices such as a lake in a paintings film. In these cases Grygar et al. suggested as a promising way to achieve the characterization, a preliminary hydrolysis with cold H_2SO_4 followed by evaporation and further analysis of the dry residue.[21]

Among all the analytical techniques suitable for the determination of organic dyes in historical artefacts, the chromatographic techniques have been (and continue to be) the most extensively used. Historically thin layer chromatography (TLC) has had an important role in this research field and continues to be used as useful tool for a preliminary identification of coloring agents due to its immediacy. [22,23]. In the last twenty years TLC has been gradually replaced with liquid chromatography (LC) equipped with ultraviolet-visible detectors (mainly diode array detector), mass spectrometers and, to a lesser extent, fluorescence detectors. [24]

The pioneer in the using of High Performance Liquid Chromatography system for the analysis of dyes in textiles has been Jan Wouters in far 1985. [25-29] Ever since, the HPLC becames the reference technique for dyestuffs analysis and still today is the most widespread in laboratories worldwide. In respect to the detection system, as predictable UV-VIS detectors are very widespread due to their cheapness, simplicity in data interpretation and natural applicability to the target compounds.

In 1995 Prof. Koren published a novel scheme for the analysis of plant and animal red, blue and purple dyes by means of HPLC. According to the author the method improved the separation and reached levels of identification of mixtures never achieved before. [30] A few years later Novotná et al. successfully applied reversed-phase HPLC to the identification and quantification of anthraquinone and naphthoquinone dyes in historical textiles. [31] In 2003 Cristea et al. published a monographic paper on the identification of the main flavonoids present in weld with a new method optimized to give a more sensitive quantification of the compounds. [32]
In the same year Orska-Gawryś et al. succeded in indentify a number of natural dyes, including alizarin, purpurin, luteolin, apigenin, carminic acid, ellagic acid, gallic acid, laccaic acids A and B and indigotin, in a series of Coptic textiles dated from 4th to 12th Century AD. [33]

In 2006 a series of publications demonstrated the growing interest and diffusion of HPLC-DAD technique and at the same time the versatility of its applications. Surowic et al. proved that dyestuffs can be detected even in fabrics not apparently colored. They studied a set of 81 archeological textiles samples from Scottish Highlands and Islands' peat bogs and detected traces of dyestuffs in 36 of them. Despite they didn't succeed in identify the exact sources of the dyestuffs because of the wide-spread occurrence of the compounds found, this study represent a first important proof of the undiscovered potentialities of HPLC analysis. [34]
Blanc et al. analyzed the natural dyes contained in historical maps belonging to The Royal Chancellery Archives of Granada. [35] Balakina et al. detected alizarin/purpurin and carminic/kermesic acid (from madder plant and cochineal insects respectively) in red wool fibers collected from cloths of Pazyryk culture found in a frozen burial of Altai Mountains (500-200 B.C.).[36]

Between 2006 and 2011 Karapanagiotis et al. published the results of the study of different archaeological findings coming from the monastery of Xeropotamou in Mount Athos. They recurred to the HPLC-DAD technique for the analysis of organic dyes in Byzatine and post Byzantine icons, and in historical textiles. They identify cochineal, dyer's broom, fuchsine, indigo, carmine, old fustic, soluble redwood, weld and young fustic. [37, 38] Dates back to 2009 the investigation on colorants used in icons of the Cretan School of iconography, carried on by the same research group by analyzing 13 icons attributed to 15th-17th century. The study revealed the use of kermes (Kermes vermilio Planchon), cochineal madder, soluble redwood and indigoid dyes.[39]

In 2009 two monographic investigations were published: Deveoglu et al. carried on an interesting study about the pigments obtained from Buckthorm berries (specifically from Rhamnus petiolaris Boiss) [40]; Cuoco et al. published the first results of their research on Madder species (Rubia tinctorum and R. peregrine). They identified alizarin, purpurin, lucidin, rubiadin and pseudopurpurin for aglycones and, lucidin primeveroside, ruberythric acid, galiosin and rubiadin primeveroside on the base of retention time and UV spectra in comparison with pure standards. [41,42]

In the same years Degano et al. achieved the detection of natural dyestuffs in pre-Columbian funeral cloths from the Peruvian necropolis of Ancon (indigo, madder, cochineal, relbunium, tannins and laccaic acids)[43], and in 16th century silk tapestries belonging to Quirinale Palace in Rome (coccid dyestuffs, madder, weld, young fustic, safflower, tannins and an indigoid dye)[44].

Despite of its wide employ and the successful result obtained, the UV-VIS detection presents several serious limitations that exclude to consider it as a decisive detection technique. The main limitations reported are low sensitivity and selectivity and inability to identify unknown compound without a proper spectral library. Since 2003 starts to be published scientific works exploring the potentiality of Mass Spectrometric detection applied to dyestuffs analysis. [46,47]

In 2008 Rosemberg published a complete review on the characterization of natural organic dyestuffs of historical interest by Liquid Chromatography - Mass Spectrometry. He discussed the structures of the most important natural organic dyestuffs traditionally used and their analytical determination with focus on the mass spectral fragmentation patterns of the different classes of dyestuffs. [48] In the same year Rafaelly et al. demonstrated the superiority of MS detectors in respect to the UV-VIS ones, by proposing a new optimized method for the analysis HPLC-ESI-MS. They successfully applied the technique to the identification of components from a small sample of wool dyed with madder (Rubia tinctorum) and Our Lady's bedstraw (Galium verum). [49]

The following year Zhang and Laursen confirmed the power of High-performance liquid chromatography (HPLC) with photodiode array and mass spectrometric detection in characterizing plant or animal dyestuffs on the basis of three orthogonal properties: HPLC retention time, UV–visible spectrum and molecular mass. They focused their investigation on yellow dyes, usually more difficult to distinguish one from the other due to the similarity of their UV-VIS spectra. They successfully analyzed historical silk fibres dyed with Sophora japonica (pagoda tree) and Curcuma longa (turmeric), and a variety of art object including a yellow varnish from a 19th century Tibetan altar and a 3000-year-old wool mortuary textiles, from Xinjiang, China.[50]
Another contemporary publication proving the effectiveness of the combined DAD-MS detection is that of Marques et al. which succeeded in detecting weld (Reseda luteola L.) and spurge flax (Daphne gnidium L.) in seventeenth century Arraiolos historical textiles [51]

A few years later Nowik et al. exploited the advanced in dyes characterization to investigate slightly soluble brominated indigoids from Tyrian purple. In contrast with the prior studies on the same target, they achieved narrow and symmetric peaks thanks to a specific method optimized to improve indigoid solubility in HPLC system. They reported, for the first time, the presence of brominated and unbrominated indirubins in purple from Hexaplex trunculus. [53]

A very recent publication by Manhita et al. reports the characterization of Arraiolos carpets' palette conducted by means of HPLC with diode array and mass spectrometry detection. Weld, indigo, spurge flax and brazilwood were identified as natural dye sources, as described in the Arraiolos historical dyeing recipes. [55]

Nowadays the most powerful technique for the characterization of natural dyestuffs is High Performance Liquid Chromatography coupled with Diode-Array and High Resolution single stage or tandem Mass Spectrometric detectors (HPLC-HR-MS). This technique combines speed with high resolution and reproducibility, and guarantees a more reliable identification of the peaks due to the combination of retention time, UV-VIS spectra, exact Mass and isotopic distribution in the MS and MS/MS spectra. Despite its undeniable advantages, very few works have been published to date.

The firsts to report application of a MS/MS detector for the identification of dyes in historical textiles were Petroviciu et al. In 2010 they successfully characterized a selection of commercial standards and a number of reference dyed wool fibers provided by different European research institutes. All the analysis were performed in HPLC system coupled to a Ion Trap mass analyzer interfaced with Atmospheric Pressure Electrospray Ionization (ESI) ion source working in negative mode. In 2012 they applied the methodology to 17th- to 18th-century Romanian textiles, three religious embroideries and two brocaded velvets demonstrating that it is a very valuable tools for dye identification in small-scaled samples. However, they specified that to better exploit the potentiality of the technique, a deep knowledge on the dyes and their biological sources must be previously acquired by standard dyes and standard dyed fibers analysis.[56, 57]

Starting from 2010, the Spanish Cultural Heritage Institute (IPCE) counts with an HPLC-Q-ToF-MS devoted the analysis of dyestuffs in historical samples. In the last years the analytical protocol developed at IPCE facilities has been successfully applied to the characterization of flavonoids, anthraquinones, indigoids and tannins in a number samples from ancient textiles and art objects including the 16th century *Códices Matritenses* by Fray Bernardino de Sahagún, where have been detected Indigotin and indirubin as component of the Mayan Blue pigment. [58,60]

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3. INTRODUCTION TO THE TECNIQUES

3.1 INTRODUCTION TO CHROMATOGRAPHIC TECNIQUE

The general term Chromatography identifies a set of laboratory techniques for the separation and further identification of complex mixtures. More exactly we should speak of preparative chromatography when the ultimate goal is to achieve a separation of the various components, and of analytical chromatography when it is also designed their chemical characterization. Actually there are several chromatographic techniques differentiated for method and equipments used but based on the same theoretical principles. The main ones are summarized in Tab. 3.1. The main concept behind the technique is the possibility to differential partitioning the various analytes composing a mixture between a stationary insoluble phase, and a mobile phase moving over it. In all chromatographic techniques, the sample is dissolved in a convenient mobile phase, which can be a gas, a liquid or a supercritical fluid. The mobile phase has the task of transporting the mixture throughout the stationary phase which can be placed in a column (column chromatography) or on a solid surface (planar chromatography). The various constituents of the mixture move along (eluite) the stationary phase length at different speeds depending on their partition coefficient (the ratio of the amounts, or better concentration, of a substance distributed between two immiscible phases at equilibrium).

These speed difference results in a differential retention and in the space-temporal separation of the compound mixture. In particular, the analytes which present more chemical affinity for the mobile phase will move quicker and come out of the column first, while those that show greater affinity for the stationary phase will come out later. In this way a separation of various analytes based on their migration velocity can be achieved.

The column is generally connected to a flow detector which gives a signal proportional to the concentration of the outgoing analytes, which depend on the property selected for detection (flammability, UV transmission, refraction index variation, mass, etc.). By plotting on a Cartesian system the detected signal as a function of time, a graph (chromatogram) in which each peak corresponds to a specific compound (or to a set of compounds in case of co-elution) can be obtained. A chromatogram can give both qualitative and quantitative information; by comparing the retention time (which identifies a well defined chemical under the same separation conditions) of the peaks with that of known reference standard analysed at the same conditions, it is possible identify them. The quantification can be obtained through the integration of peaks (calculation of the peak area).

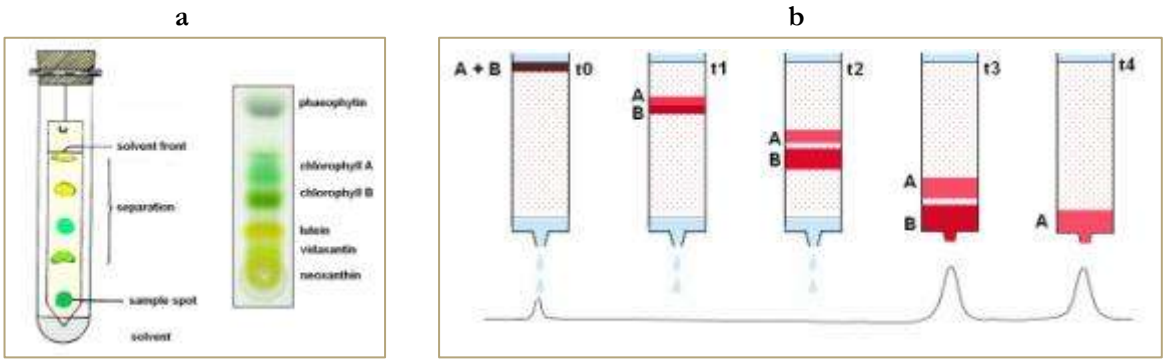

Fig.3.1 - elution mechanism in a TLC paper (a) and a chromatographic column (b)

TYPE	METHOD	STATIONARY PHASE	EQUILIBRIUM
GC - Gas Chromatography	Gas-liquid	Liquid adsorbed onto a solid surface	Partition between gas and liquid
	Gas-solid	Solid	Adsorption
LC - Liquid Chromatography	Liquid-liquid	Liquid adsorbed onto a solid surface	Partition between no miscible liquids
	Liquid-solid	Solid	Adsorption
	Ionic exchange	Ionic exchange resins	Ionic exchange
	Size exclusion	Liquid entrapped in the interstices of a polymeric solid	Sieving
	Affinity	Liquid with specific groups bonded to a solid surface	Partition between a liquid film and the mobile fase
Supercritical fluid chromatography		Organic compound bonded to a solid surface	Partition between a supercritical fluid and a surface

Tab. 3.1 – Main chromatographic techniques with relatives equilibria

3.1.1 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

As has been said, the chromatographic technique adopted in this study is the High Performance Liquid Chromatography chromatography (HPLC) that is actually one of the most applied to the analysis of such kind of substances.

HPLC chromatography is derived from the classical liquid chromatography (LC), the first studied chromatography, but presents a number of advantages compared with it. In old LC the eluent moves along the column due to the gravity or to the low pressure generated by the volume of liquid, while in HPLC the mobile phase is forced to flow within the column by an high pressure generated by a pumping system. This change allows to considerably shorten time analysis and to dramatically increase the number of theoretical plates and, consequently the separation resolution and efficiency. The apparatus consists of four basic

elements: The pumping system, the injection system, the column and the detector. Fig. 3.2 reports a block diagram of a normal instrumentation for HPLC analysis.
The following paragraphs briefly illustrate the singles modules composing an HPLC equipment.

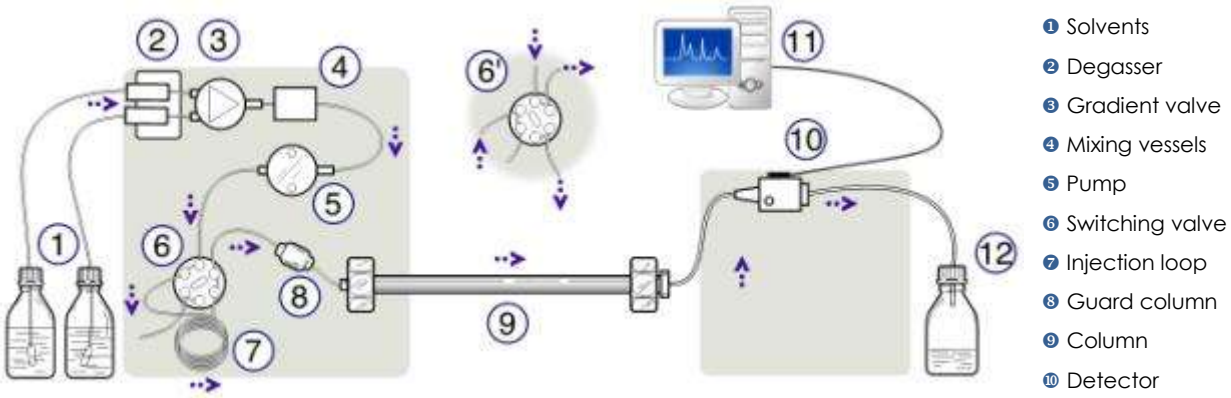

Fig. 3.2 - Simplified scheme of a standard HPLC system

PUMPING SYSTEM

The pumping system is one of the most important elements of the HPLC apparatus since by its efficiency depends the success of the analysis and the column lifetime. An HPLC pump must have several features: must be able to generate pressures up to hundreds bar (>1000 in most recent systems) , must guarantee a constant and reproducible eluent flow (as low as 0,001µL/min in most recent nano-HPLC pumps) usually between 0.1 to 10 mL, and must have a good resistance to solvent corrosion. There are three types of pumps that are commonly used: Reciprocating pumps, syringe screw pumps and constant pressure pumps. The majority of modern chromatographic equipment mounts a reciprocating pump that has numerous benefits including a reduced internal volume (50 to 250 µL), a higher maximum pressure, an easier adaptation to gradient elution and a good robustness.

INJECTION SYSTEM

Two different methods exist to introduce the sample into the chromatographic apparatus: the stop-flow method and the sampling loop. These have almost completely replaced the traditional syringe loading in which the injection was executed directly performing with a syringe a septum made of elastomeric material. This method, in fact, does not guarantee reproducibility and is inapplicable at high pressures (>50 bar). In the stop-flow injection, the solvent flow is stopped to allow the removal of a small amount of solvent and to avoid the sample loading in the column head.

Nowadays the most used loading method is that based on a 10 ports valve system (sampling loop). Thanks to this valve, the sample is introduced upstream the column and is dragged into it by the mobile phase without interruption of flow. Almost all current HPLC systems today can be equipped with autosamplers providing the automatic loading of the samples and guarantying much higher reproducibility and precision in the injection of the desired volume.

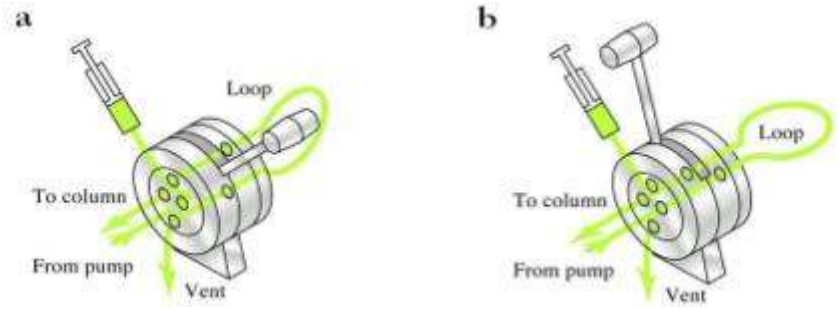

Fig. 3.3 - Sample loading (a) and injecting (b) in a sampling 6 ways loop valve

COLUMN

The analytical columns for liquid chromatography are generally constituted by a stainless steel tubes or polymeric tubes measuring 2-40 cm in length and 1-5 mm of internal diameter, even if smaller column (down to 10 μm) are currently available. The stationary phase packing is composed by small particles of porous silica or polymeric material having diameter generally ranging from 1,5 to 5 μm. With such columns is possible to achieve a fairly high number of theoretical plates, approximately from 40000 to 100000 theoretical plates per meter of length. The column is generally preceded by a guard-column that has the function to remove particulates and other impurities that may be contained in the solvents. The use of guard column facilitates the analysis and largely extends the columns lifetime. In some cases they also serves to saturate the mobile phase with the stationary one in order to minimize the possibility of loss of the stationary phase by the analytical column.

DETECTORS

The detectors for HPLC can be divided into two large categories: those selective towards a specific class of analytes (e.g. only the fluorescent compounds) and the universal ones, that reveal (theoretically) all components (e.g. mass spectrometer). Among all the possible detectors the more frequently used in liquid chromatography include the UV-VIS detectors, the fluorescence detectors, the refractive index detectors and, above all mass spectrometric detectors (available at reasonable prices only in the last 20 years). The mass spectrometer is the most powerful tool coupled to a HPLC separation system because it can provide an

accurate identification of analytes by separating the ions according to their mass-charge ratio (m/z). These detectors consist of two basic elements: The ionization system (source) and the analyzer. The source has the task of fragment and ionize the analytes trough different techniques. Various source are available according to the ionization technique applied: EI Source (Electron Impact), CI (Chemical Ionization), FAB (Fast atom bombardment), ESI (ElectroSpray Ionization) and MALDI (Matrix Assisted Laser Desorption and Ionization)
Similarly, there are several types of analyzers differing for the charges manipulation mode (by means of a magnetic field, an electric field, or application of a continuous and alternating potential) and for the parameters used for the detection of the m/z signal. The mass spectrometric analyzers currently available on the market are resumed in Tab. XXX. There are also hybrid mass spectrometers obtained combining two or more mass spectrometric devices ('in space' tandem MS. They avoid tandem mass spectrometry, an advanced technique based on the based on consecutive re-fragmentation and analyses of the analysed ions so that to obtain MS^n fragmentation patterns. It should be pointed out that some single device are already designed for such kind analysis ('in time' tandem MS). These, as well as the most common tandem configurations, are resumed in Tab. 3.2.

SINGLE DEVICES		
Magnetic sector mass spectometer (B)	MS	
Electric sector mass spectometer (E)	MS	
Quadrupole mass filter(Q)	MS	
Ion trap mass spectometer	MS^n	
TOF - Time of flight mass spectometer	MS	
Orbitrap	MS^n	
QqQ - Quadrupolo-Quadrupolo	MS/MS	
TANDEM DEVICES		
BEqQ hybrid mass spectrometer	MS/MS	a magnetic sector (B), electric sector (E), collision quadrupole (q) and m/z selection quadrupole (Q)
BEBE - four sector mass spectrometer	MS/MS	
QTOF - mass spectrometer (also QqTOF)	MS/MS	Quadrupole coupled with time-of-flight
QqQ - Triple quadrupole mass spectrometer	MS/MS/MS	

Tab. 3.2 – Mass Spectrometric Analyzers employed in HPLC

3.1.2 OVERVIEW ON THE QUADRUPOLE – TIME OF FLIGHT MASS SPECTROMETER COUPLED WITH API-ESI INTERFACE

In ESI ionization systems, the sample, dissolved in a polar solvent, is sprayed (at atmospheric pressure in most recent instruments) in a ionization chamber through a needle held at a high electric potential. The droplets of spray, which are positively charged by the action of the electric field, are dried by a gas flow (usually nitrogen) and the free ions are then attracted towards a "ions extraction lens", which is constituted by a charged glass capillary maintained under vacuum. In this way the solvent evaporates and the charged ions are accelerated toward the analyzer. The applied voltage is usually set in order to maximize the charge z to one, even if z>1 can be preferentially formed in larger mass ions, such as protein and peptide fragments.
In the practice a high electrical potential is applied at the end of the column to so that drops are formed more gas flow tangential to the column to obtain the spray (the Venturi effect). Then you experience the following factors: the solvent evaporates, the droplets become smaller, the positive charges, being closer, no-explode and you will get individual ions (Coulomb effect). With these processes, starting from a liquid, ions are obtained at atmospheric pressure and room temperature.

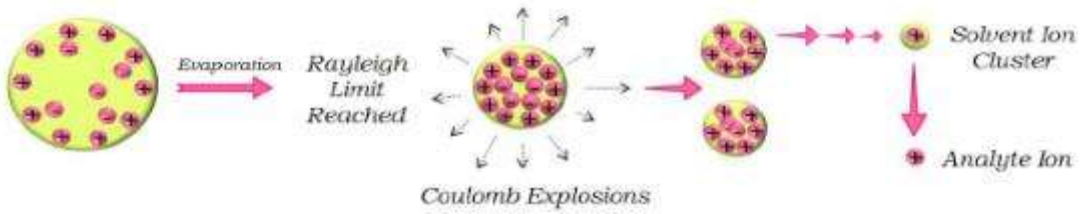

Fig. 3.4 - mechanism of ions formation in a ESI interface

The Q-TOF or QqTOF is an hybrid tandem mass spectrometer composed by a quadrupole analyser and a Time of flight analyser separated by a collision quadrupole.

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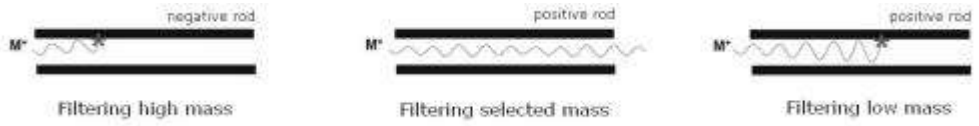
Quadrupole mass spectrometer - The quadrupole mass analyzer is composed by four parallel metal rods that are electrically connected in pairs (each rod is connected to the opposite one). Proper direct current (DC) and radio frequency (RF) voltages can be applied to the bars in order to filter out all ions except those of a particular m/z value. The RF is applied to all four rods, but the negative "-" rods are 180 degrees out of phase with the positive "+" rods. The rods are labelled + and – because of the fluctuating DC voltage which is applied. The composite voltages on both pairs of rods are equal in magnitude and opposite in sign. All ions generated at the source travel down the

quadrupole between the rods, but, for a given voltage value and frequency, only a certain m/z value of ions may reach the detector (only those that fit the equation $m/z=[k'\Omega^2r^2]V$), while other ions are obliged to follow irregular and unstable trajectories and will finally collide with the rods. In particular higher m/z ions will drift into the negative rods because the RF force is not strong enough to overcome the ions momentum while lower mass ones will be accelerated into the positive rods when the rods have a negative voltage. This avoid the selection and further analysis of a specific ion (SIM, Selected Ion Monitoring), or permits to execute a full MS scan for a range of m/z values by continuously varying the applied voltage



Time of flight mass spectrometer - The TOF analyzer is a straight tube of 50-100 cm at the end of which is applied an electric field that accelerates the ions coming from the source. The ions, generated in the source and flown toward the detector by a charged plate, are then forced to pass through a straight field-free region. At the end of the flight all the ions are subjected to the same electric field and consequently take the same kinetic energy.
Since Ek = ½ mv2, on equal kinetic energy conditions, the heavier ions (with a greater m/z ratio) move slower than the lighter ones (with smaller m/z ratio), and require more time to reach the detector. Taking advantage of this principle the "time of flight" of the various ions can be used to measure their m/z ratio.

$$\frac{\boldsymbol{m}}{\boldsymbol{z}} = \left(\frac{\boldsymbol{2V}}{\boldsymbol{L^2}}\right)\boldsymbol{t^2}$$

where **V =** voltage applied in acceleration, **L=** flight path and **t =** time of flight

The mass is directly calculated by the management software which apply several correction including a primary calibration based on the equation $m = a(t - t^0)^2$, the reference mass correction (correct **a** and $\mathbf{t_o}$ based on know masses in the spectrum of interest) and the systematic deviations correction (higher order polynomial to fit arbitrary residuals).

In tandem mass spectrometry the ions created by the ionization source pass through a first analyzer that act as a mass filter selecting a specific m/z ion called precursor ion. The precursor ion is then selectively fragmented in a collision cell and all generated fragments are sent to the second analyzer to be detected and identified.

In a QTOF system the first quadrupole has the function to select a ion of interest among the whole coming from the source, The second quadrupole, also known as collision cell, focus the ions and forces their collision and subsequent fragmentation by introducing a collision gas (the process is called CID, Collision Induced Dissociation). The Time of Flight mass spectrometer analyze the fragment ions with high accuracy, sensitivity and resolution.

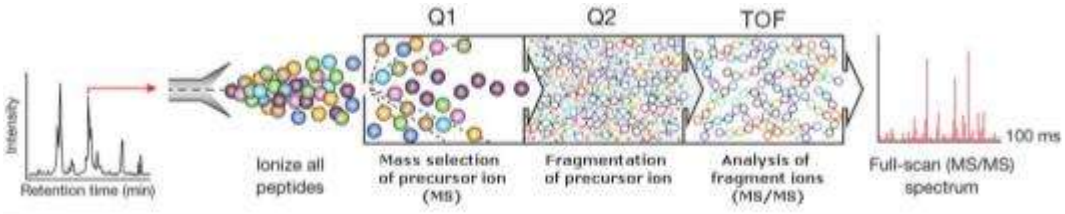


Fig. 3.5 - mechanism of fragmentation and analysis in space tandem mass spectrometry

The resulting fragmentation pattern is characteristic of the molecule but can vary depending on the fragmentation energy. At lower energies (close to the threshold), fragmentation reactions are often limited to losses of neutral molecules (H_2O, MeOH, CO, CO_2 etc.) that are usually structurally little significant, although they can supply information about functional groups.

At higher energies retro-synthetic type reactions can occur. These are much more structurally significant, and often result in cleavage of the molecule at characteristic positions. High care mast be given in CID since an excess energy transfer can lead to uncontrolled fragmentation such as cleavage of simple C-C bonds.

Until a few years ago TOF Mass Spectrometers had a quite modest (< 3000) resolution and sensitivity but were anyway very efficient in the study of synthetic polymers and biological macromolecules due to their relatively wide m/z range (up to 3000). For proper operation, however, had to be coupled to sophisticated pulsed ionization systems such as the MALDI source. The current systems are actually real high resolution (up to 50000) spectrometers that ensures great analytical reliability. The main advantages of the Quadrupole-Time of Flight Mass Spectrometer as detector for HPLC systems can be summarized as follow:

- extended mass range (about 30-4000 m/z) that allows the analysis of a very wide range of molecules, from solvents to proteins.
- simultaneously identification along the entire m/z range (50-2000 usually);
- excellent mass accuracy (<1-20 ppm) produced thanks to an high resolving power;
- capacity to identify component even in unresolved chromatographic peaks, even in narrow ones, due to its high speed data acquisition;

- capacity to reveal low and high concentration analytes, due to its exceptional sensitivity and wide dynamic range;
- increase of the signal/noise ratio due to the grouping of tight ions (increases of peaks height);
- very quickly acquisition system.
- Possibility to use either the quadrupole or TOF analyzers independently or together for tandem MS experiments.

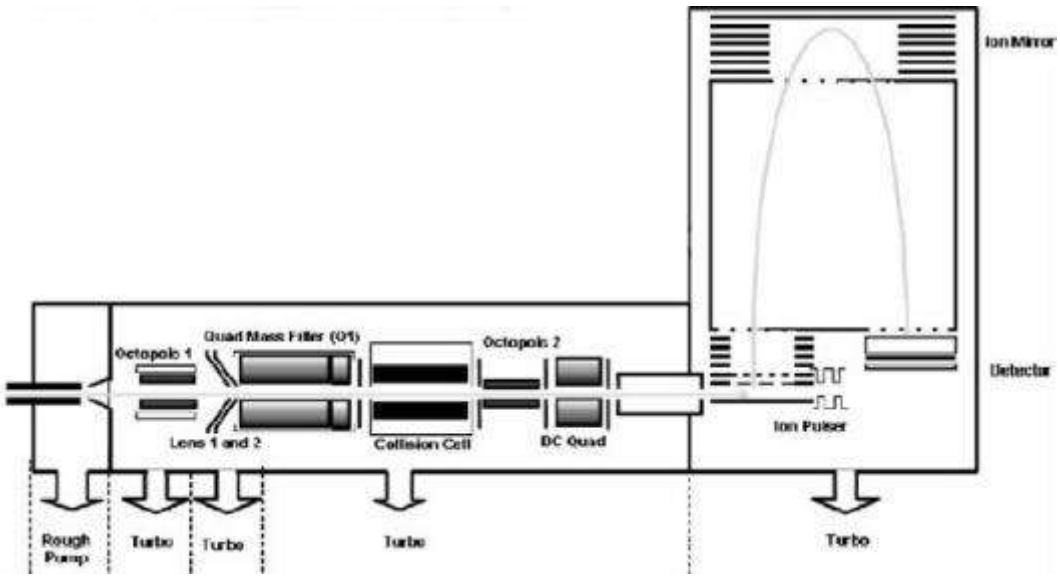


Fig. 3.5 - mechanism of fragmentation and analysis in space tandem mass spectrometry

4. PRE ANALYTICAL PHASE

4.1 PREPARATION OF THE LAKES

4.1.1 CHOICE OF THE HISTORICAL SOURCES

Among of all the documental sources initially considered a selection of that emerged as more representative or exhaustive for the thesis purpose has been done. All the applied recipes belong to the Bolognese Manuscript (*Segretti per colori*, XV century), the Marciana Manuscript (*Segreti d'arti diverse nel Regno di Napoli*, XVI century), the Paduan Manuscript (*Ricette per fare ogni sorte di colore*, XVII), the Brussels Manuscript (*Recueuil des essaies des merveilles de la peinture*, XVII century) and the Jehan Le Begue collection. As can be seen, the treaties cover a lapse of time of seven centuries, ranging from the early Middle Age to the late seventeenth century

- *Segretti per Colori* is a collection of recipes compiled in the second quartet of Fifteenth century by an unknown author. The text, entirely wrote en early Italian and Latin, is a precious source since it contains detailed information about materials and procedures different crafts (painting, dyeing, ceramics etc.). *"It is a book of recipes than a treatise, and affords interesting notices of all the decorative arts practised at that period in Bologna."* It is better known as Bolognese Manuscript since the only original copy is conserved in the Bologna University library (cod. MS Lat.2861).

- The Marciana Codex is a heterogeneous collection of recipes concerning pharmacology, cosmetics, gastronomy and of course arts and crafts. "*It is a collection of recipes which make us acquainted with many compositions of the old professors, used in medicine, surgery, farriery, chemistry, painting, illuminating, gilding, working in stucco, varnishing, and similar works".* It was edited in 1570 in Gaeta area and give a overview on the southern Italy artistic techniques. Due to its thematic variety, the Merrifield chose including just an extract of the Manuscript. The full text is still conserved in the Marciana library of Venice (cod. It. III.10).

- The Paduan Manuscript is a anonymous venetian text dated back to the last decade of XVI century or, more probably to the middle of the XVII[1]. It is written in Italian with the exception of section No.83 that is in Latin. It contains prescriptions and recipes mainly concerning the art of illumination.

[1] "The handwritting is of sevententh century , and althoug, from the following circumstances, the MS may have been written during the latter part of the sixteenth century, I think it more probable that it was composed during the middle, of latter part, of the seventeenth century." Mary Philadelphia Merryfield, *Original Treatises dating from the XII to XVIII Centuries on the Arts of Painting, London, John Murray, Albemarle Street, 1849 ,VOL II, pag. 643*

- The manuscript of Jehan Le Begue consists in a number of late medieval texts dealing with art and craft techniques. Le Begue took charge to copy these manuscripts in a single codex in the 1431 and prepare. The collection include fragments of *Schedula diversarum artium* by Theophilus, the Manuscript of Eraclius (*De coloribus et artibus romanorum*), the Manuscript of S. Audemar (*Liber Magistri Petri de Sancto Audemaro de Coloribus Faciendis*), and the Manuscript of Archerius (*De Coloribus Diversis Modis Tractatur* and *De Diversis Coloribus).*

- *The Brussels Manuscript (Recueil des essais des merveilles de la peinture*) is a texts written by the painter Pierre le Brun in 1635. It is believed that the author wrote the manuscript during his stay in Paris and that the treatise could represent an overview of Seventeenth French painting technique. The text is a sort of glossary redacted with the goal to give to amateur painters the terms and the knowledge to speak on the subject with property. It is preserved in the Brussels public Library (cod. 15.552)

*A*ll these manuscripts are contained in the *Original Treatises dating from the XII to XVIII Centuries on the Arts of Painting*, the collection of ancient manuscripts redacted by Mary Philadelphia Merrifield. The volume was published in 1849 as a result of a nine years research activity throughout Italian libraries and Institutions. In 1840 the historian was charged by the Britannic govern with the important task to trace and transcript ancient manuscripts dealing with artistic techniques. Her collection represents a very important tool to anyone who wants to approach the study of past Art and Crafts techniques.

MANUSCRIPT	TITLE	AUTHOR	AREA	DATE
Bolognese MS	*Segreti per colori*	unknown	Northern Italy	15th c.
Marciana MS	*Segreti d'arti diverse nel Regno di Napoli*	unknown	Southern Italy	16th c.
Paduan MS	*Segreti per fare ogni sorte di colore*	unknown	Northern Italy	17th c.
Brussels MS	Recueil des essais *des* merveilles *de la peinture*	Pierre le Brun	France	17th c.
Jehan Le Begue M.	*Schedula diversarum artium*	Theophilus	Northern and central Europe	15th c.
	De coloribus et artibus romanorum	Heraclius		10-11th c.
	De Coloribus Faciendis	Petrus de Sancto Audemaro		13-14th c.
	De coloribus diversis modis tractatur	Archerius		14th c.

Tab. 4.0.1 – List and specifications of the manuscript sources consulted during the research activity

4.1.2 TROUBLES IN SOURCES INTERPRETATION

During the preparation of lakes some interpretative difficulties have been encountered. The first obstacle to a immediate comprehension of the texts has been the language in which they are written. The manuscripts are in fact written in an Old Italian language rich of dialectal inflections, in Old French, in Latin or in an Italian-contaminated Latin. This problem is partially solved by the English translation given by Mary Merrifield, notwithstanding it should be taken very carefully due to the numerous inaccuracies and inaccuracies. Furthermore, in evident that the historian encountered the same difficulties at the moment to translate the manuscripts and there are large portions of text remained untranslated.
The main difficulty related to the language is the presence of words that are fallen into disuse or disappeared, or words whose significance has changed in the course of centuries. Furthermore it must be remembered that at the time the most manuscripts were written, do not existed an Italian language yet, therefore a lot of the employed words are more properly belonging to regional dialects. Hereinafter is reported a glossary with the most common critic words and the relative translation.
The second, but not less important problem, has been the almost absence of objectives doses in the recipes. Many of them describe with extreme accuracy the procedures without mention the quantity of ingredients required to realize them (e.g. "*quanto te paia sia bastevole*"). In a number of recipes the doses, although present, are approximated (e.g. a fistful, three fingers etc.) or expressed in ancient unit of measure. Even in the case of known units, the conversion of such quantity to the current metric system is quite hard due to regionally dependence of the single units of measure. Formerly each region, or in many case each city, had an own metric system and even the same unit could assume different values (fortunately usually with a negligible gap) from a city to another. Regarding the time measurement, the most recipes used well known prayers (e.g. *spatium trium miserere*) to define time periods. Clearly it implies a not objective evaluation of time depending from the velocity at which they are pronounced. The main units of measurement founded in the recipes are the following:

bocale: container with a locally variable capacity; 1,3 l in Bologna, 1,1 l in Florence and Modena, 0,8 in Milan.
***broco, brocha*:** carafe
dragma: dramma, weight unit corresponding to 1/8 of oncia
foglieta: container with a capacity of half a litre
libra: pound. Weight unit corresponding to approx 340 grams
mezeta: volume unit corresponding to approx 0,57 litres
metatella(rum), metadelle: container with the capacity of a mezzetta
oncia: weight unit corresponding to approx 28 grams (1/12 of libra)
otava: eighth part of a higher unity
pinta: northern Italy volume unit corresponding to 1,3 - 1,5 litre (XIV century).
quatrini: weight unit corresponding to 1/5 of oncia
scrupuli: weight unit corresponding to 1/24 of oncia
terzarulo: container and volume unit for liquids, third part of other unit

GLOSSARY WITH THE MOST COMMON CRITIC WORDS:

***admisia*:** to mix

***albio*:** earthenware container

alumj de feccia: Cream of tartar, $KC_4H_5O_6$

aluminis roccj, aluminis rocze, aluminis rozi, alumj de rocho: potash alum, KAl(SO4)2.12(H2O)

aluminis scaiolj: lamellar gypsum, $CaSO_4 \cdot 2H_2O$

alumj zucharino: alum ground and heated with sugar, rose water and white of egg, and then dried by cooling.

amistalo: mix it

aqua gomata: water containing arabic gum

aq(uae) artentis: wine spirit

aqua tartari: Cream of tartar water solution

arap(re)sa: coagulated

arsenico ch(rista)llino: Arsenic compoung obtanined by heating orpiment (As_2S_3) and rock salt (NaCl)

arzica: yellow dye from weld (reseda luteola)

assa fetida: latex extracted from ferula assa-foetida

besiche: bladders

biacha, biaca: lead white, $2PbCO_3 \cdot Pb(OH)_2$

biacca cruda: raw lead white

brasilium: red dye from Brazilwood

callo: decrease, loss

cals, calx: slaked lime, $Ca(OH)_2$

camilli(n)a: Brown-colored, camel-colored

capitellum: very concentrated lay, K_2CO_3

capitem foratam i(dest) miscolam: slotted spoon

cenere da vetrio: alkali-based flux for making glass

cenere de feccia: Cream of tartar ash

cenigem, cinige: hot ash with sparks

ceruse: lead white

ciato vitrj: glass cup

cimatura de grana, cimatura de rosato, cimatura d(e) scarlacto de grana: ritagli del tessuto con conniniglia

liscia, lisciva: lye, ranno, v. lexivium.

liscivo capitis: lye used to hair washing

luto de sapie(n)tia, loto: a mixture of clay with arabic gum, lime and other ingredients, used to seal

mele, melle: honey

mele rosato: honey with rose extract

mescola, misculam: ladle, dipper

miscola, mistica: mix

cinerem fetie: ash from wine dregs, containing K_2CO_3

citositatem: lemon juice

coccia, cocia, coculeam, cocleam:: mollusc shell

coclareo: spoon

colatoro: colander, strainer

colla de branch(e): leather scraps glue

collofonia, colofonie: colophony, greek pitch

com(m)arabico: Arabic gum

co(n)cha: eartenware container

coppi: roof tiles

cremusinus: scale insect, cochineal

crevellare, crebellata, crebellatis: to sift with a sieve

cribello: sieve

dragantj: tracanth,gum extracted from Astragalus species

endico, endicum: indigo

fecce: dregs, sediment formed on the barrels bottom after fermentation of the grape must

gem(m)o (sale): rock salt, NaCl

ghebbj: Plant with blue dying seeds. Not translated. Maybe Sambucus ebulus or Mirabolanus chebuli.

giallate: evaporated, exhaled

gilosia: Amaranthus tricolor

gomarabico: Arabic gum

grana: Red dye extracted from cochineal

gualda (herba): Weld, Reseda luteola

guatj tintoru(m), spuma(m) indici sive guatj: dyers wood, isatis tinctoria,

guato fiore: flower of woad, the best part of woad

gum(m)am lacce: shellac

indicat(a): indigo coloured water

indicum de bagadon, indico, indicum: oriental indigo

i(n)finentre: while, meantime

lacha: lake

lavella: basin, washbowl

lexivium lesciviu(m): lye, ranno

scrillent(e): clear, transparent

scurla: shake, agitate

scutella: bowl

salis alcalj, sale alchelj: Sodium of Potassium carbonate

sandolj: sandalwood, Pterocarpus santalinus

sanguine draconis aut lacca: dragon's blood

mordente: mordent, mordanting agent.
morella: plan of solanacee, Solanum nigrum L.
oculos pulcinj: blue flowers plant, Veronica persica
ornello: Fraxinus ornus L.
pagnolo: small pot, cauldron
panicella, herba roccia: Weld, Reseda luteola L.
Pelliparij, piliciarj: furriers, tanners
pero citrino: pear variety, thus called for its yellow fruits
pignatto: cooking pot
pignolato: linen fabric with pinenuts-like decorations
pila: mortar
ragia pini, ragina, rasina: pine turpentine
raich(e): roots
rama de fico: fig branch
ranno: lye
rogello: orchil, Roccella tinctoria L.
saguinarella o herba spagnola: Galium aparine L. or Polygonum aviculare L., formerly called bloody grass
sal netrio, salis nitrij: salpetre, potassium nitrate KNO_3
scotano: Smoke tree, Rhus cotinus
scutes: cup
smolglio: lye
spinj cervinj, spingerbino: Rhamnus catharticum
spregne(n)do: squeezing
spogna spongna, spo(n)gia: sponge
stacia: sieve
stamegna: cheesecloth, cloth for filter liquids
tartaro: Cream of tartar, $KC_4H_5O_6$
tasum, tasi albj: white cream of tartar
t(er)bentine: turpentine
t(er)ra bianca: furriers' clay
testas ovo(rum): eggshells
untijs: ounces
venacia, venatia: marcs
v(er)zinu(m) sive brasilium,virzino: Brasilwood
vitrioli romanj: Roman vitriol, iron sulfate, FeO_4S
vittollo(rum): from egg yolk
zaffaramj, zafferamj: saffron, Croccus sativus L.

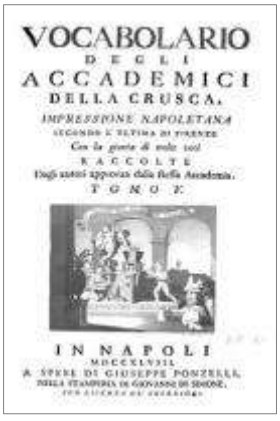
DICTIONARIES AND SOURCES CONSULTED:

[1]

[2]

[3]

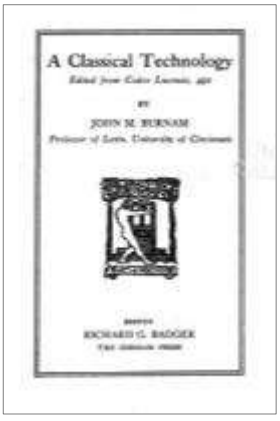
[4]



[5]

[6]

[7]



[8]

4.3 PRELIMINARY PREPARATIONS

Before starting with the synthesis of the organic pigments, the preparation of some basic formulations has been necessary. These ingredients are mentioned in almost all the recipes and has been prepared and stored to be used during the entire experimental period. They includes lye, synthetic urine and alumj zucharino.

4.3.1 PREPARATION OF LAY

The lay is an alkalin solution obtained by boiling water with wood ash. Due to its desgreasing properties, it was used in ancient times as domestic detergent, expecially for the cleaning and whitening of textiles. The cleaning properties of the mixture are due to the potash contained in the ashes in the form of carbonate. In the dyeing field was employed as alkalin extraction media. The lay used in this study was prepared as follow:

1. A part of wood ash has been accurately sifted in order to eliminate all the carbon particles. This step is really important in case of preparation of a lay to be used for dyes extraction because, if not removed, the unburned particles gave a dark colour (from brown to black depending on the amount) to the solution.
2. The cleaned lay has been put in a large Pyrex container and mixed with clear water in a ratio of 1:5 by volume (1 part of ash and 5 of water). The eventual carbon particles still presents in the ash have been removed by removal of the supernatant layer.
3. The ash/water mixture have been heated to boiling point in a laboratory hot plate and then cooked during two hours over moderate heat stirring once in a while.
4. Once reached the desired pH (≈ 9-10) the mixture has been removed from the fire and let stand for a day, in order to cool down and let the ash deposits in the bottom of the container.
5. The carbonate formed on the air-lay interface has been removed and the clear lay was separated from the ash and transferred in a glass bottle.

Fig. 4.0.1 Preparation ol lye from wood ash

4.3.2 PREPARATION OF SYNTHETIC URINE

The preliminary survey of the manuscripts showed that a number of recipes required urine as extraction medium. Urine has always been employed in the dyeing industry as proved by historical sources and archaeological findings. It had a variety of applications, starting from the preliminary cleaning and softening of the fibres, to arrive at the extraction and fixing of dyestuffs. In the present work, to solve the problem of urine supply, the use of synthetic urine has been carefully considered and finally adopted.
In research activities the employ of synthetic urine represents a valid alternative to the more problematic use of the natural one. It provides a matrix that reproduces the environment of the urine and that at the same time is completely devoid of pharmaceuticals or other potentially adulterant substances that may be present in a real urine matrix.
The synthetic urine was prepared according to the Kark's procedure (Kark et al. 1964), following the recipes published on "The American Biology Teacher journal (More 1994). The complete list of reagents required to prepare 2 l of artificial urine is detailed in Tab. 4.2.

COMPOUND	FORMULA	CAS NUMBER	AMOUNT
Distilate water	H2O		1,5 l
Urea	NH_2CONH_2	57-13-6	36,4 g
Sodium chloride	NaCl	7647-14-5	15 g
Potassium chloride	KCl	7447-40-7	9 g
Sodium phosphate dibasic dihydrate	$Na_2HPO_4 \cdot 2H_2O$	10028-24-7	9,6 g
Creatinine	$C_4H_7N_3O$	60-27-5	4 g
Albumin		9048-46-8	100 mg

Tab. 4.0.2 - Recipe of synthetic urine used in the study

1. In a Pyrex Becker 1.5 liters of distilled water have been mixed with 36.4 g of urea until complete dissolution of the crystals. 15.0 g of sodium chloride, 9.0 g of potassium chloride and 9.6 g of sodium phosphate have been weighted and admixed to the solution. The solution has been stirred until it became clear.

2. The pH of the solution has been checked with an indicator paper to ensure it was within the pH range expected for normal human urine (5 to 7). Since it was quite out of range the pH have been adjusted by adding of hydrochloric acid 1N.

3. The thus prepared solution has been stored in fridge until the moment to use it. Before use it, it was warmed to room temperature and then admixed with 4.0 g of creatinine and 100 mg of albumin. These two compounds ensure a greater similarity to human urine.

4.3.3 PREPARATION OF ALUMJ ZUCHARINO

The name "Alumj zucharino" identifies an alum-based formulation widely employed for the preparation of lakes and other pigments. According to ancient sources [1-6] it was obtained by heating alum with rose water, sugar and white of egg. The solution thus obtained was then hardened by cooling. Since

1. First of all, the rose water has been prepared as follow. The petals of one natural (not treated) red rose have been put in a large Becker and covered with approx 300 ml of distilled water. The water containing the petals has been gently heated up to boil, and then boiled during 15 minutes (or until complete discoloration of petals). The rose extract has been filtered and transferred into clean glass vials.

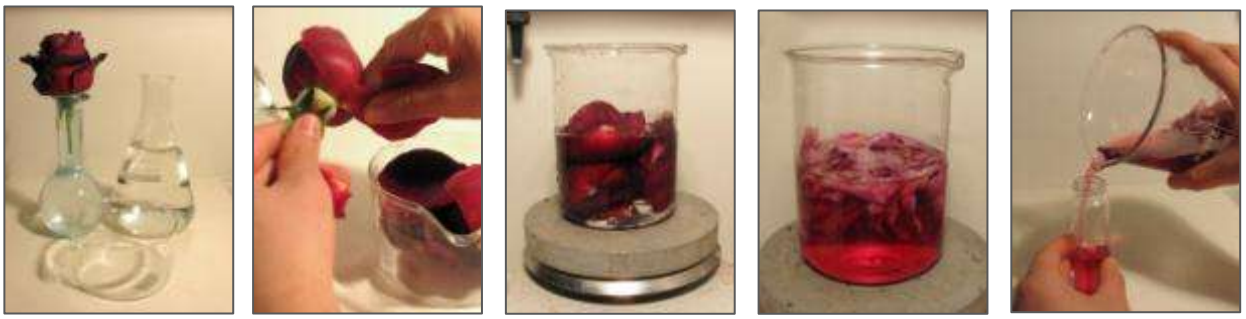


Fig. 4.0.2 – Preparation of rose water

2. Since none of the sources reported doses or a specific procedure for the preparation of the "Alumj zucharino", the mixture has been done following a personal choice. The white of an egg has been beaten with a fork until achieve a more liquid consistency.

3. Three teaspoons of alum finely grinded has been admixed with one teaspoon of sugar, two teaspoons of egg white, and enough rose water to obtain a pasty consistency. The formulation has been heated during 20 minutes in a hotplate and then let cool at room temperature. The hard crust obtained has been grinded in a ceramic mortar.

Fig. 4.0.3 – Preparation of Alumj zucharino

4.4 PREPARATION OF LAKES

4.4.1 MATERIALS

All the dying species (berries, barks, flowers, roots, leaves, insects etc.) and the mordants used in the preparation of organic pigments were from Kremer (Kremer Pigmente GmbH & Co. Aichstetten, Germany), except some fresh product (e.g. Iris flower, Solanum nigrum berries and leaves etc.) that have been direct collected in the countryside. For the sake of completeness, some commercial dyestuffs and lakes have been included in the study. Hereinafter is reported the list of all the dyeing material employed.

RAW MATERIALS


Alkanna
Kremer 37250


Brazilwood
Kremer 36150


Buckthorn ripe
Kremer 37380


Buckthorn
Kremer 37390


Campeche
Kremer 36100


Cochineal
Kremer 36040


Frangule
Kremer 38520


Gamboge
Kremer 37050


Saffron
Commercial


Iris germanica
fresh


Lac
Kremer 60430


Madder
Kremer 37199


Old fustic
Kremer 36200


Rhatany
Kremer 37260


Safflor
Kremer 37420


Weld
Kremer 36250


Weld
Fresh


Woad
fresh

DYESTUFFS AND LAKES


Lac dye
Kremer 36020


Madder lake
Kremer 37202


Indian yellow
Kremer collection


Cochineal
Mexico


Cochineal
Perú


Tyrian purple
Kremer 36015


Indigo
Kremer 36003


Indigo
wildcolours


Mayan Indigo
wildcolours


Tamil Nadu Indigo
wildcolours


Woad
Kremer 36003


Woad
wildcolours

MORDANT AND ADDITIVES

4.4.2 METHOD

More than 200 recipes have been experimented during the research activity, not always successfully. In many case the replication of the recipes resulted in the mere extraction of the colorants without formation of an insoluble precipitate. This could be attributed to a bad interpretation of the texts or to a wrong dosage of the ingredients (see par. 4.1.2 for further details about interpretative troubles).

Table 4.3 (see next page) summarize the nineteen tinctorial species investigated in the study and contains the list of pigments prepared and analyzed for each of them. The samples identified by the prefix MB, MM, MP, MJ and MBr indicate that the receipts derive from the Bolognese Manuscript, the Marciana Manuscript, the Paduan Manuscript, the Brussels Manuscript and the Jehan Le Begue collection respectively. Those beginning with the prefix KR are commercial lakes supplied from Kremer (Kremer Pigmente GmbH & Co. - Aichstetten, Germany). This phase has required approximately an year of laboratory activity among interpretation of the treatises, supply of materials and enactment of the recipes.

Among the whole experimented recipes, five extraction techniques have been identified on the base of the procedure and of the extraction medium. They can be resumed as follow:

1. Direct release of the dye in the binding media (e.g. white egg, arabic gum)
2. Extraction in an acid solutions (e.g alum water, vinegar, lemon juice)
3. Extraction in alkali solutions (e.g. lay, slaked lime solution)
4. Extraction in neutral of low acidity/alkalinity solutions (e.g. water, urine, wine)
5. Extraction from dyed textiles ("*cimatura*" or "*pezzette*")

Hereinafter, an example for each extraction technique is provided. All the other experimented recipes are detailed in annex I.

SPECIE	DYEING SPECIES	COLOUR	SAMPLES
Alkanna	Alkanna tinctoria	purple	ALK1, ALK2, ALK3, ALK4, ALK5, ALK6
Brazilwood	Caesalpinia echinata	Red	MB112, MB116A, MB116B, MB118, MB121, MB123, MB127, MB130A, MB130B, MB130C, MB132, MB133, MB136 A, MB136B, MB136C, MB203, MBrVII12, MBrVII14, MBrVII20, MJ101, MJ108, MJ17, MJ181, MJ299, MJ304, MP115A, MP115B, MP115C, MP77, MP77A, MP77B, MP87
Safflower	Chartamus tinctorius	yellow	CAR1, CAR2, CAR3, CAR4, CAR5, CAR6, CAR7, CAR8, CAR9
Fustic	Clorophora tinctoria	yellow	CLO1, CLO2, CLO3
Cochineal	Coccus cacti	Red/purple	Coc1, MB110, MB111, MB137A, MB137B, MB139A, MP116, CoMex, CoPer
Saffron	Crocus sativus	yellow	MB184, MB234, MJ 337, MP128, MP 93
Gamboge	Garcinia hanburyl	yellow	MP19, KR2
Campeche	Haem. campechianum	purple	MP87C, CAM1, CAM2, CAM3, CAM4, CAM5, CAM6
Indigo	Indigofera tinctoria	blue	KR 3
Iris	Iris germanica	green	IR1, MB96, MP15, MP65, MP86, MP112
Woad	Isatis tinctoria	blue	KR5, MB75G, MB76G, MB77G, MB79, MBRVII9, MP63
Lac	Kerria Lacca	red	KR7, MB140, MJ100, MP113
Tyrian Purple	Murex brandaris	purple	KR8
Weld	Reseda Luteola	yellow	MB135A, MB135B, MB135C, MB135D, MB135D, MB194A, MB194B, RES1, RES2, RES3, RES4
Sap green	Rhamnus cathartica	Green/yellow	KR1, MB92, MP29A, MP29B, MP31, MP79, MP89, MP105, MP133, MP137
Frangula	Rhamnus frangula	Brown/orange	FRA1, FRA2, FRA3, FRA4, FRA5, FRA6, FRA7, FRA8, FRA 9
Rathany	Rhamnus ratania	Brown/orange	RAT1, RAT2, RAT3, RAT4, RAT5, RAT6, RAT7, RAT8
Madder	Rubia tinctorium	RED	KR9, RB1, RB2, RB3, RB4, RB5, RB6, RB7, RB8
	Solanum nigrum	GREEN	MB91, MJ37

Table 4.3. list of the analyzed samples

1

BOLOGNA MANUSCRIPT, RECIPE N. 70

Ad colorandum azurrum

Reccipe verzinum et subtiliter rade cum vitro et pone rasuram illam in clara ovi preparata per diem et noctem ita quod rasura illa sit coperta a dicta clara cum modico aluminis rocci pulverizati deinde colabis hec omnia cum petia panni lini alba et cum predicta clara colorata temper- abis azurrum.

Take some scraped verzino and put it into prepared egg white (1,2) for a day and a night (3). It must be completely covered with the egg. Add a little Potash alum (4) and strain the mixture with a piece of linen. Use the coloured liquid to distemper blue pigments.




2

BOLOGNA MANUSCRIPT, RECIPE N. 89

Affare verde bono cum spincerbino

Reccipe granellj de spino gerbino quando sonno bene mature et metili in uno vaso de vetrio et amalpalj bene cum le mano et metilj al sole et lassalj stare tanto che leve suso li grappi e quelle venacie poi la cola et premilj bene et gieta via quella venacia et grappi et se lo dicto sugo fusse una libra metice doi quatrinj dalumj de rocho spolverizato poi lo pone al sole in vaso de vetrio bene serato et lassalo stare 3. o 4. dj et omni di lo mistica 3. o 4. volte molto bene atorno et per spatio de tempo se secasse distemperalo cum ranno da capo chiaro cum uno poco di gomma

Put some ripe berries of buckthorn in a glass vase and press with finger in order to disrupt them. Place the vase under the sun and let it remain until the juice cover the berries. Strain the juice pressing well the marc and add 1,3 g of potash alum per every 100g of juice. Place the mixture in the sun in a closed vase for three or four days stirring well 3 or four times every day. To use it after long time, distemper with clear ley with a little gum.



3 BOLOGNA MANUSCRIPT, RECIPE N. 132

A fare laccha per altra forma

Tolli verzino raso cum vetrio o cum la raspa la quantita che tu voli. £t se la raditura fosse pieno uno bichiero tolli la mita de lo dicto verzino et polio da canto et l'altra mita micti a molle in tanto ranno da capo che lo verzino stia bene ooperto dalo dicto ranno et lassa stare a moUe per spatio dmia nocte poi lo pone a buUire al foco temperatamente et commo ha bulito per una ave maria et tu tolli de quello verzino che reservasti et mettivini supra a quello che bolli a poco apoco et cusi continua per infinj che nai sempre staendo imo pooo da una volta alaltra et commo non nai piu et che dicto verzino sia arentrato per mita et tu tolli tanto alumj de rocho qnanto te pare bastevilj et metivilo dentro et mistalo uno pocho et sia bene spolverizato et subito poi lo leva dal foco et lassalo reposare bene et fredare bene poi lo cola per panno de lino raro solamente quello che uesce da se senza aspremare le fece de niente. (...)

Take one-half vase of Brazil wood and put it to soak in strong lay. Let it rest for a night. Put the solution to boil slowly over the fire for a space of a *Ave Maria* (1) and then add the same quantity of other Brazil wood. When the solution is reduced to one-half (2), add a little powdered alum and stir. Take it away from the fire and let it rest and cool down. Filter (3) and let it dry in the sun for a day or two (4, 5). To have a darker pigment add a little lime when the solution is boiling.




4 BOLOGNA MANUSCRIPT, RECIPE N. 121

Affare el verzino al fuoco

Tolli mezaonciade verzino raso subtile poi tolli tanto vino bianco quanto copra el dicto verzino poi lo pone in uno pignatello vitriato novo et lassalo mollare per spatio de uno di naturali poi tolli una otava dalumj de rocho et altratanto gommarabico spolverizato poi lo pone in lo dicto pignatello dal verzino et lassalo stare un altro di poi lo pone a bullire al foco et quando sera arentrato permita poi lo lassa fredare poi lo cola cum una peccia de panno di lino et serbalo in ampolla de vetrio bene turata et sera bono

Put 13 g of brazil wood in a glass vase and cover it with white wine (2). Let soaking for a day (3) and then add 1/8 of potash alum (4) and the same amount of powdered Arabic gum (5). Let it stand for another day. Boil until the liquid is reduced one-half. Cool down, filter and preserve the in a closed glass bottle.





5 BOLOGNA MANUSCRIPT, RECIPE N. 110

A fare laccha bona et bella

Tolli lb j de cimatura de grana de rosato e mectila in ranno fortissimo facto de cenere la quale usa li tentori in una pignatta yitriata nova et poUa al fooo a bullire et bolla pianamente per spatio de doi pater nostri poi mecti el ranno et la cimatura per uno collatoro netto de panno de lino et premilo forte cum mano siche tutto el ranno escha fora et poi repone el dicto ranno a bullire de novo senza ala cimatura et bolito el gieta sopra ala cimatura che e in lo collatoro et preme forte el colatoro cum mano siche tutto el ranno escha fora bene et ripollo da parte et la cimatura gietta via et lava molto bene il colatoro siche non 1^ rimaoga veruno pelo de la ditta cimatura poi tolli once cinque dalumi de rocho spolverizato subtili et metilo a poco a poco per vdta in el dito ranno per infino che el ranno se strenge che lo conoscirai quando el dito ranno tutto quasi se converti in una achinma grassa in fino al fondo et mai se vole finare de mistare el dicto ranno cum uno cochiaro netto (...)

In a glass vase boil for the space of a "pater noster" 4,50 of cochineal clippings (1) with a strong ley (2). Put the mixture in a strainer covered with a linen cloth and press with finger. Let the ley boil again and then strain it in the containing clippings strainer . Add slowly 1,3 g of powdered potash alum until a thick scumm grow up (3,4). Stirr the mixture until it became cold. Strain with a linen cloth (5) and let the lake dry. Wash it with fresh water in order to remove all the scumm and let it dry again in the shadow (6).







4.5 PREPARATION OF PAINTING MODELS

A set of reference coatings have been prepared. They include two oil painted tablets, two tempera painted tablets and a set of microscope slides containing both the coatings (one slide for each lake).

The tablets, destined to imaging techniques and to sampling, have been realized such a way as to reproduce the structure of a real painting. The priming layers (preparazione and imprimitura) have been prepared according to the Cennino Cennini's prescriptions in regard to the realization of panel painting. The application of a canvas has not been necessary since a commercial tablet have been employed. First of all the priming binders have been prepared by mixing rabbit glue and water in 1:7 (v/v) ratio. The glue has been let swell during 12 hours before being cooked in a bain-marie until complete dissolution. The thus prepared glue has been admixed with Bologna chalk until achievement of the desired consistency (quite pasty). A thick layer of Bologna chalk has been applied and let it dry a few days. Once completely dried, the surface has been smoothed out using a moderate grain sandpaper (320 meshes) in order to remove all the coating imperfections. A second thinner coating has been finally realized by application of three layers of Bologna chalk admixed with a larger quantity of glue. It has been decided to apply the same *imprimitura* layer both to the oil and the tempera tablet because the oil/lead white coating, more appropriate in case of oil paintings, would have required too much time to dry out. Once completed, the prepared surface has been divided into sections by drawing 3 x 1,5 cm slots with a soft pencil. The oil tablet has been realized by admixing the lakes with pure linseed oil while for the tempera one a previous step has been required to prepare the binder. Once again the Cennino's treatise has been consulted to the egg tempera recipe. The binder has been prepared mixing an egg yolk with half an eggshell of clear water. Until being mixed with the water the yolk has been deprived of any trace of albumen by cleaning under a mild water flow and removing of the external cuticle. A teaspoon of vinegar has been finally added as anti-fermentative agent.

The microscope slides, destined mainly to microscopic investigation, have been executed realizing the brush strokes directly in the glass surface. The binding media were the same used in the tables.

4.6 AGENING OF REFERENCE SAMPLES

Since early Nineteenth century a lot of studies have been focused on understanding the problem of fading, that is the main degradation process occurring in dyestuffs and dyed materials. Principal responsible of this alteration process is the light exposure as reported in many referenced works. Due to their nature, organic dyes are the perfect targets for photochemical reactions, light induced oxidative reactions that could take place just if molecules absorbed light. Exist three basic photochemical reactions:

1. The dye can absorb light passing to the instable excited form and then decompose. In this case the photodecomposition does not need other compounds to take place.

   1) $D \rightarrow D^*$ 2) $D^* \rightarrow$ decomposition products

2. The dye absorb light passing to the excited form but is instable, and consequently decompose, just if other substances are present in the system. These substances react with the excited dye molecules, thereby converting them into other compounds. In absence of other compounds in the systems the activated dyes are reconverted in the stable ground states by physical deactivation processes. (no fading)

   1) $D \rightarrow D^*$ 2) $D^* + A \rightarrow$ reaction products

3. In addition to the dyes other compounds present in the system absorb light and pass to the excited form and them react with the dyes.

   1) $A \rightarrow A^*$ 2) $A^* + D \rightarrow$ reaction products

4. In the system is present a photocatalyst that absorb light and reacts in is activated state with the dye molecules or with other substances present. In this process the dye is destroyed and the photocatalyst regenerated.

   1) $C \rightarrow C^*$ 2) $C^* + D \rightarrow C$

In the firsts two cases the photoactive compound is the dyes while in the third and in the fourth is the compound A and the catalyst respectively. It means that just in the first two mechanisms the action spectrum of the reaction is determined by the absorption of the dyes. The most wide spread mechanism is the second one by oxidation of the secondary compound and simultaneous reduction of the dye.

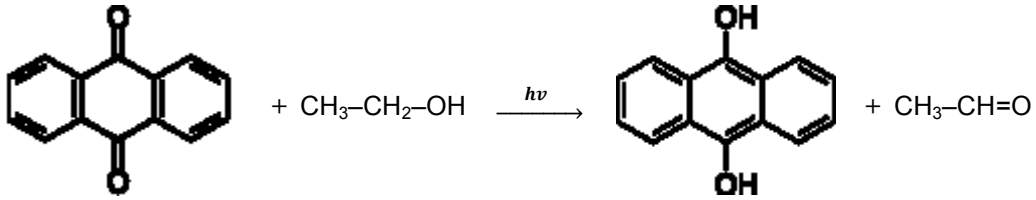


Fig. 4.4. Example of reaction between anthraquinone and ethanol. The photo-activated anthraquinone is reduced to 9,10-dihydroxyanthracene

In general light induced degradation rate of organic pigments depends on the intensity and the spectral distribution of the radiation to which they are exposed. In general the rate of color change is usually proportional to the length of exposure. Earliest studies on the topic shown that fugitive dyes are more sentitive to visible radiation, while dyes of high light fastness are faded mainly by UV radiation (McLare 1956)(Gantz and Sumner 1957).

In addition to the light many other factors contribute to the fading of natural dyes. We can distinguish them into two big category: external and internal factors. Among the external ones the most important are undoubtedly humidity and temperature that could accelerate the degradation processes and increase the fading rate of the dyestuffs. A definitive explanation about the relation between fading phenomena and humidity/temperature conditions as not been given yet. Nevertheless the most probable explanation is that an high moisture in the system increases the diffusion of reagents thus facilitating the reaction. Studies aimed at the reduction of fading processes in historical dyed textiles have demonstrated that a drastic reduction of relative humidity (up to 25%) bring to a significant reduction of fading.

The internal factors are those characteristic of the own system. The mains are the nature, the concentration and the physical state of the dyestuffs, the nature of the matrix and/or substrate (e.g. fibers, underlying paint layers etch) and the mordant type. Since the oxidation-reduction reactions are prevalent, dyes which contains oxidant functional groups (e.g. carbonyl or quinonic groups) are easily subjected to fading. Several studies demonstrated that the physical state is more important than chemical structure in the determination of fading processes. More finely dispersed dyes are mostly (and more quickly) subjected to fading. In respect to the dyes concentration exist an inverse proportionality relation since the fastness of a dyestuff increase with increasing of dye concentration. However all the factors contributes to the fading it has been demonstrated that the ones that mainly affects the degradation of dyes are mordant nature and mordanting method

4.6.1 MATERIALS AND METHOD OF THE AGING PROCESS

Both the paint's model tablets and the powder pigments have been subjected to accelerated aging in order to reproduce as possible the composition of real samples. The final aging has been achieved in two stages: 8 month of natural aging followed by 12 of artificial aging. Natural aging has been conducted between May 2012 and January 2014. During the whole period the tablets have been placed in front of a window facing East and exposed to natural sunlight with the only filter of the window panes.

Artificial aging has been conducted into a Binder KBF-P 240 (E 5.2) constant climate chamber (Binder GmbH - Tuttlingen, Germany). The device allows the control of temperature and humidity within a range of 10-70°C and 10-80 % rH respectively. It is equipped with three bright white lamp and two Synergie Light™ fluorescent tubes that ensure homogeneous lighting conditions. The illumination system is conformed to the ICH (International Conference on Harmonisation) guidelines in regard to photostability testing (Q1B). All the Climatic chamber specifications are detailed in Tab. 4.4.

The climatic operative parameters have been set to a temperature of 50 °C at 60% relative humidity. To guarantee a good exposition of the powder pigments to the illumination source during the aging, a multi-cell glass plate has been specifically designed.

Climate data (with humidity)		**Temperature data (without humidity)**	
T range without illumination cassettes (°C)	10 - 70	without illumination cassettes (°C)	0 - 70
T range with illumination (°C)	10 - 60	with illumination (°C)	10 - 60
T variation with illumination		Max. heat compensation up to 40 °C with illumination (W)	400
at 25 °C and 60 % rH (± K)	0,6		
at 40 °C and 75 % rH (± K)	0,6	**Illumination data**	
T fluctuation with illumination		ICH - compliant illumination device for photostability testing (Lux) / (UVA W/m²)	8.000 / 1,2
at 25 °C and 60 % rH (± K)	0,2		
at 40 °C and 75 % rH (± K)	0,2	**Electrical data**	
H range without illumination cassettes (% rH)	10 - 80	IP protection class acc. to EN 60529	IP 20
H range with illumination (% rH)	10 - 75	Voltage (± 10 %) 50 Hz (V)	200-230
H fluctuation with illumination		Nominal power (Kw)	2,4
at 25 °C and 60 % rH (± % rH)	1,5		
at 40 °C and 75 % rH (± % rH)	2		

Tab. 4.4 Climatic chamber specifications

Fig. 4.5 – Interior view of the operating climatic chamber

4.6.2 AGING ASSESSMENT

Fig. 4.6 - tempera paint model of red/blue lakes before (left) and after (right) artificial aging

Fig. 4.7 - oil paint model of red/blue lakes before before (left) and after (right) artificial aging

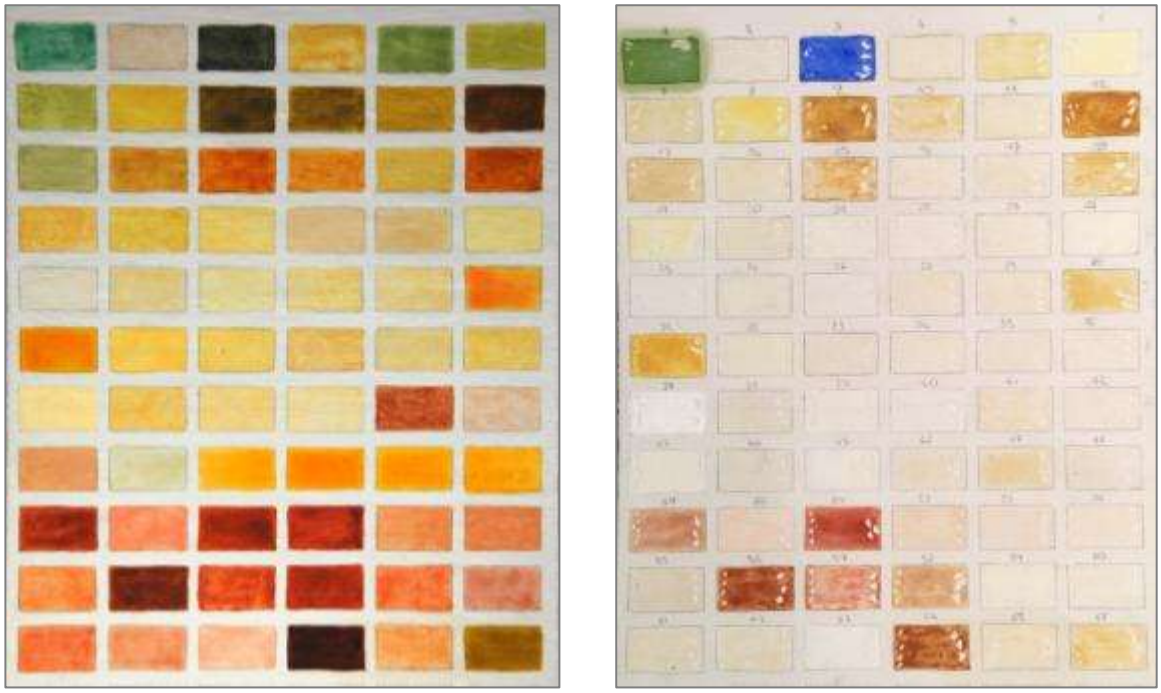


Fig. 4.8 - tempera paint model of green/yellow lakes before (left) and after (right) artificial aging

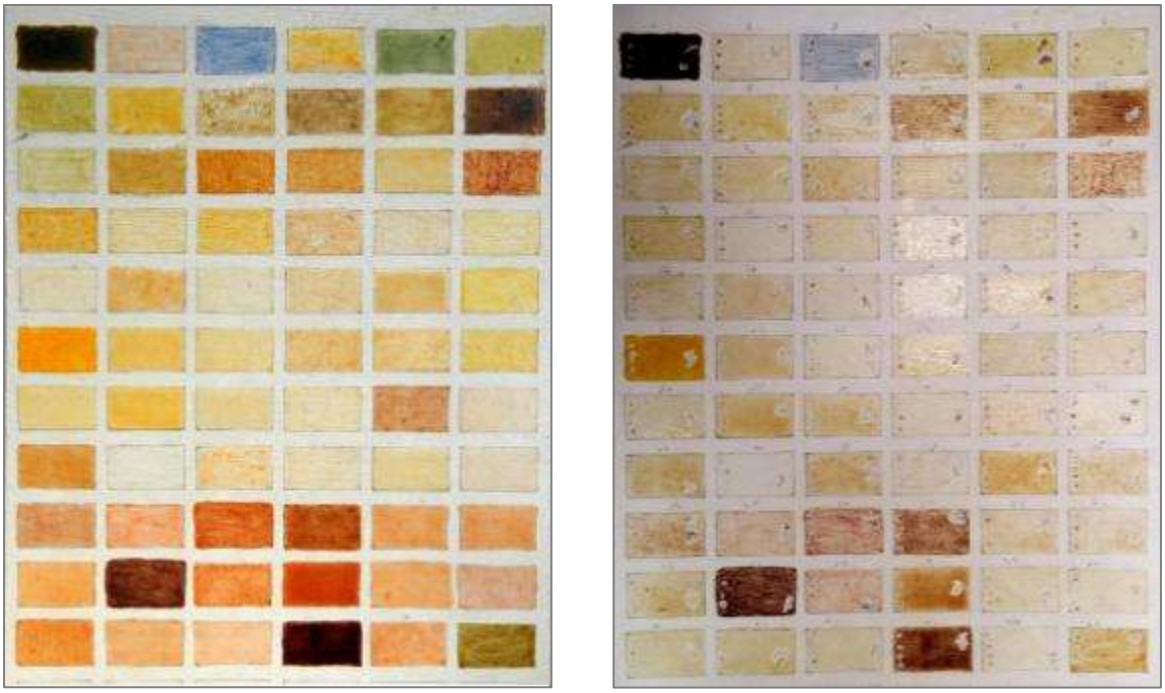


Fig. 4.9 - oil paint model of green/yellow lakes before (left) and after (right) artificial aging

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5. EXPERIMENTAL SECTION

5.1 HPLC COUPLED TO Q-TOF-MS AND UV-DAD DETECTORS

5.1.1 REVIEW ON EXTRACTION METHODS

Prior being analyzed by means of HPLC-Q-ToF-MS all the samples have been subject to a pre-analytical treatment in order to extract the organic analytes from the solid pigments and dissolve them in an aqueous/organic solvent. The extraction step is crucial, within the whole analytical method, because all the consideration about the sample (e.g. identification of dyestuffs) will be grounded on the extracted components. In case of lakes or mordent dyes a preliminary de-complexation treatment is always necessary in order to break the coordination complex and free the dyes molecules (analytes) from the metal ions.

The standard procedure proposed by Wouters and Verhecken 25 years ago (Wouters, Verhecken 1989) is still today a widely adopted method for the solubilisation of anthraquinonic dyes (and more in general of mordant dyes). It consists in the treatment with a hydrochloric acid/methanol/water 2:1:1 (v/v) solution for 10 min at 100°C. The efficacy of the method is based on the formation of chlorine complexes and on the dissociation of phenolic group with consequent suppression of their bonding action. According to the original method the dry residue is then dissolved in H_2O:MeOH (1:1, v/v). This step is very efficient in the extraction of anthraquinones and flavonoids but absolutely not in case on the indigoids that are insoluble in such solvents. To improve the recovery of indigoid dyes some authors proposed an additional extraction step with DMF, MeOH/DMF (1:1 v/v) (Surowiec, Quye et al. 2006), or DMSO. Although the good extraction yields obtained with this procedure, the treatment with a strong acid results too aggressive for labile compounds. Under the effect of hot hydrochloric acid the glycosides are subjects to the hydrolysis of glycoside bonds, causing that only the aglycone molecules can be detected. As mentioned previously, glycosides contributing to the composition of a lot of natural dyestuffs (especially yellow and red dyes), therefore their failure identification leads to an important loss of information.

In the attempt to overcome these limitations several investigations have been carried out and some mild procedures have been proposed. Even though each of them uses a different pathway to dissociate the colorants from their complexes, all the methods are based on a common approach: a complexing agent (e.g. F- ions, EDTA or HCOOH) competing with the dyes for the metallic ion (e.g. Al^{3+} in case of alum mordanted lakes).

In 1995 Tiedemann and Yang (Tiedemann, Yang 1995) developed a milder procedure consisted in treating the sample with 0.1% N_2EDTA in H_2O/DMF (1:1, v/v) at 100°C during 30 min in a boiling bath and then cooling it rapidly. According to the authors this

method was as powerful as the traditional HCl method and guaranteed the preservation of the sample structure (in their case textile fibres) for further investigation.

In 2003 Surowiec et al. published a comparison study of five different treatment including ethylenediaminetetraacetic acid (EDTA), oxalic acid, oxalate, citrate and citric acid. According to the authors none of them was better than the HCl extraction although the use of oxalic acid allows the recovery of larger amounts of alizarin and carminic acid.

A few years after Zhang and Laursen (Zhang 2005) presented two different extraction protocols based on the use of formic acid/methanol 5:95 (v/v) at 40 °C for 30 min, and of 0.001 M aqueous Na_2EDTA/ACN/ MeOH, 2:10:88 (v/v/v) at 60 °C for 30 min and subsequent dissolution of the dry residue in water/methanol 50:50 (v/v). The both method gave higher yields than the HCl procedure allowing at the same time the detection of some unhydrolysed glyocosides. According to the experiments done by the authors formic acid method proved to be quite efficient in the extraction of anthraquinone-type dye while the EDTA method in the extraction of flavonoid ones.

In a personal communication at the 25th Meeting of Dyes in History and Archaeology wich took place in Suceava, Claude Andary proposed a mild method based on the use of oxalic acid 1M. (Guinot and Andary, 2006). The method, remained unpublished, was successfully applied to weld dyes wood but not to other tinctorial species.

An interesting study of 2009 (Valianou et al., 2009) compared three of the up to then knew extraction methods (HCl, citric acid, oxalic acid) with two new ones based on the use of trifluoroacetic acid (TFA) and a combination of HCOOH and EDTA. The different procedures were assessed with respect to: (a) number of compounds extracted, (b) relative quantities of compounds extracted and (c) signal-to-noise ratio of the extracted compounds.

For the TFA method the samples (1–2 mg of dyed textiles) were treated with 400 μl of 2M TFA/MeOH:H_2O (2:1:1 v/v/v) solution during 10 minutes at 100°C. The extracts were then evaporated under nitrogen stream at 65°C, reconstituted with DMSO and, once cool down, centrifuged at 4000 rpm.

For the HCOOH–EDTA method 400 μl of 5M aqueous HCOOH/MeOH:H_2O (2:1:1 v/v) were added to the samples. The tube was kept at 100°C during 5 min and then charged with 400 μl of EDTA 0.5 mM. After 5 minutes the extract was cooled, centrifugated and the upper solution was avaporated to dryness under gently nitrogen stream at 65°C. After a second extraction the residue was reconstituted with DMSO and centrifuged at 4000 rpm. The authors' experiments proved the supremacy of the TFA procedure among the five considered.

In 2011 another comparative investigation has been done to evaluate the efficiency of eight referenced procedures in the extraction of natural dyes (cochineal, madder, woad, weld, brazilwood and logwood) from wool samples. (Manhita et al., 2011)

In addition to the already described procedures (HCl/MeOH/H_2O method by Wouters, HCl + MeOH/DMF method by Surowiec, Formic acid and Na_2EDTA/ACN/MeOH method by Zhang and Laursen, Na_2EDTA in H_2O/DMF by Tiedemann and Yang, Oxalic acid method by Guinot and Andary) the authors considered two extraction procedure specific for blue indigoid dyes. The first was that developed by Schweppe in the far 1979 consisting in repeatedly boiling the sample with dimethylformamide (DMF) until it remains colourless. The second is the alkaline hydrolysis with hot pyridine proposed by Surowiec in 2003. (Surowiec et al., 2003)

All the methods thus far described have been developed for the extraction of natural dyes in textile fibers and then applied (sometimes indiscriminately) to pictorial samples. This fact contributes to demonstrate the reduced attention and insufficient research performed on the specific topic of lakes and related problems.

The only systematic study focused on the specific extraction of lakes is that carried on by Dr. Jana Sanyova at the Institut Royal du Patrimoine Artistique (IRPA-KIK, Belgium). It is a mild extraction method based on the use HF as complexing/hydrolyzing agent. According to the protocol the paint micro-sample (0,1-0,5 mg weight) is subject to a first extraction with an opportune solvent(s) (typically in ACN/MeOH 1:1 v/v) in order to swell and remove the organic binding media. The residue is treated with 20 ml of aqueous HF (4M), vortexed and left at room temperature during half an hour. Once expired the extraction time the HF solution is subject to elimination of fluorides by means of manual clean-up on SPE C18 cartridges or evaporation to dryness followed by an automated HPLC online clean-up. As proved by the author the method is widely applicable to the extraction of the more acid-resistent dyestuffs (alizarin, purpurin, kermesic acid etc) from aluminium-based substrates, to substrates with other mordants (e.g. barium, tin), to non-mordant dyes (e.g. indigo) and to synthetic dyes (e.g. Hansa Yellow, indigocarmine) (Sanyova 2008)

This method shows a great efficiency in recovering both stable and labile molecules such as glycosides and acid-sensitive compounds (e.g preudopurpurin, munjustin). Furthermore it allows in most cases the recovery of higher amount of coloring matter in respect of the traditional HCl method. As demonstrate by other mentioned procedure, the milder pH alone is not sufficient. The HF method efficiency is in fact due to the combination of two factors: the milder pH, which prevent acid hydrolysis of the precursors, and the formation of strong aluminium-fluoride complexes, which remove a large fraction of the aluminum ions present, influencing the thermodynamic equilibria and strongly reducing the H+ concentration necessary to compete with Al^{3+} ions for the phenolate groups of the dyes molecules.

5.1.2 ADOPTED SAMPLE PREPARATION PROTOCOL

The extraction procedure adopted in the present research work is that developed by Dr. Estrella Sanz Rodríguez at the *Instituto del Patrimonio Culturale de España* (IPCE, Madrid) within a collaboration in the project for "The Red that Colored the World"[1]. The method has been optimized starting from the above mentioned HF procedure (Sanyova 2008). The IPCE protocol involves a decomplexation/extraction step assisted by a moderate heating at 60-70°C. The extracting solution is composed by hydrofluoric acid 4M, methanol and milliQ water in 2:1:1 (v/v/v) ratio. As can be noticed, this step differentiates it from the Sanyova's procedure that was entirely conducted at room temperature using pure HF.
The elimination of fluorides is achieved evaporating to dryness under a weak nitrogen stream. The dry residues are reconstructed with methanol/dimethylformamide 1:1 (v/v), a solution able to bring into solution all the different colorant types including the indigoids. An effective recovery of the freed dyes is obtained with a second extraction at 80°C during 5 minutes followed by natural cooling and filtration.
With respect to the IPCE method two additional steps (see step 3 and 7) have been introduced. They consist in a brief ultrasound treatment (5 minutes each) with immersion of the eppendorf tubes in an ultrasonic bath. This practice allows a better desegregation of the sample resulting in a visible improvement of the extraction. The complete preparation procedure is schematically detailed hereinafter.

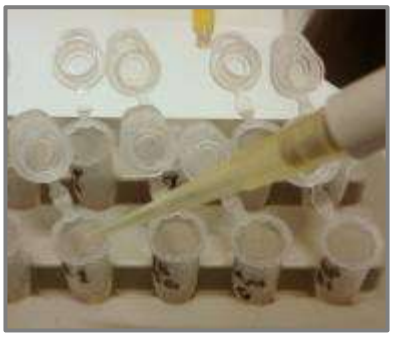
MATERIALS

The sample extraction was performed into Eppendorf® polypropylene tubes by Eppendorf AG (Hamburg, Germany). The extraction solvents, absolute grade (≥99.5%) Dimethylformamide and LC-MS Ultra CHROMASOLV® grade (≥99.9%) methanol, were both from Sigma Aldrich Co. (St. Louis, MO, USA). The water has been purified to milliQ grade level with the Direct-Q 3 UV system by Millipore (Billerica, MA, USA).
The filtration was carried on 0,2 µl nylon filter Costar® Spin-X® microcentrifuge tube by Corning Incorporated (Corning, NY, USA) using a microcentrifuge Hettich EBA 21 from DJB Labcare Ltd (Newport Pagnell, Buckinghamshire, England).
The multiblock termostastic bath and the ultrasound bath employed were both from JP SELECTA S.A. (Abrera, Barcellona, Spain). The evaporation of sample was achieved with a Techne® sample concentrator by Bibby Scientific Limited (Stone, Staffordshire, UK)

[1] "The Red that Colored the World", Museum of International Folk Art (MOIFA) Santa Fe (New Mexico, United States. Exhibition scheduled for January 2017.

EXTRACTING METHOD

Approximately 0,1 mg of each lake are weighted and put in an polypropylene tube

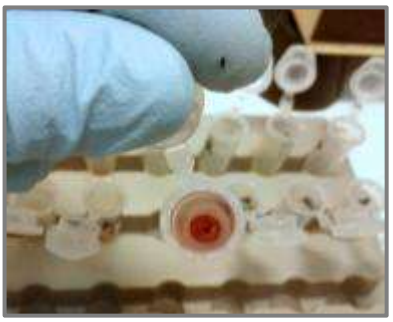


The samples are treated with 100 µl of a HF/H_2O/MeOH 2:1:1 (v/v/v) solution.

The tubes containing the solution are sonicated during 5 minutes in ultrasound bath

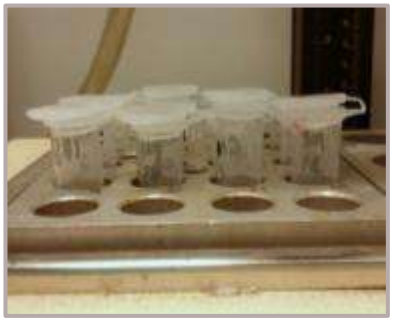
The tubes are placed in a thermostatic bath and kept at the T of 60-70°C during 20 min

The solution is evaporated to dryness under a weak nitrogen stream

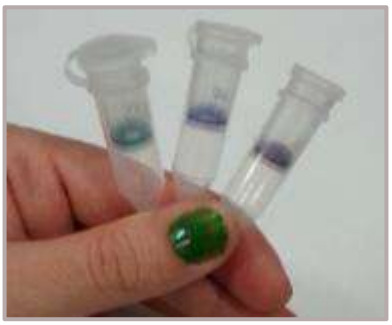


The residue is reconstructed by adding 100 µl of MeOH/DMF 1:1 (v/v)

The tubes containing the solution are sonicated during 5 minutes in ultrasound bath.

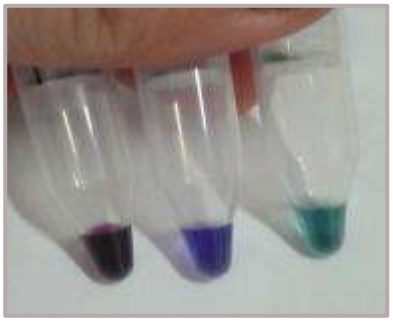


The tubes are placed in the thermostated bath and kept during 5 min at the T of 80°C

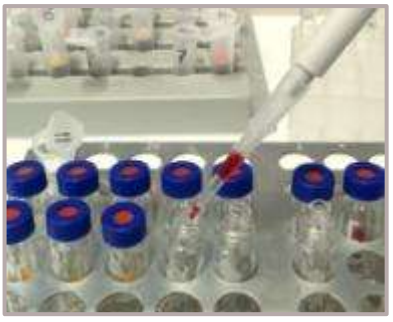


The extracts are transferred into micro centrifuge tubes with 0,2 µm nylon filter

centrifuged during 10 minutes to a speed of 6000 rpm



The extracts are put in a 100 µl insert placed in the HPLC vials



The extracts are put in a 100 µl insert placed in the HPLC vials

5.1.3 ANALYTICAL METHOD

The analytical equipment consisted in a High Performance Liquid Chromatography system equipped with a UV-VIS Diode Array Detector (DAD) and a Quadrupole - Time of Flight Mass Spectometer (qTOF-MS). The LC apparatus presented the following configuration: Autosampler , Binary pump, degasser, thermostated column compartment. All the modules of HPLC - DAD - QTOF system were from Agilent Technologies (Snta Clara,USA).
The analytical method have been developed by Dr. Estrella Sanz Rodriguez at IPCE (Instituto del Patrimonio Cultural de España) facilities. Specific chromatographic conditions and optimized MS spectrometer parameters are detailed in the following paragraphs.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The chromatographic separation was achieved using a Zorbax - C18 SB column (50 x 2.1 mm,1.8 μm particle size) as stationary phase and a binary mobile phase consisted of 0,1 % (v/v) formic acid in water (eluent A) and pure acetonitrile (eluent B). The following gradient was employed for standards and real sample analysis: isocratic elution with 10% B up to 0,4 min; linear gradient to 22% B up to 6,5 min; isocratic condition up to 8 min; linear gradient to 35% B up to 14 min and then to 95% up to 18 min; isocratic up to 21 min; linear gradient to 10% up to 25 min. Column temperature and elution flow were kept constant throughout the analysis at 35°C and 0,75 ml/min respectively. Separated compounds were detected with a 1200 Series Diode Array Detector scanning from 200 to 800 nm, monitoring at λ= 275 nm, λ= 350 nm, λ= 550 nm y λ= 600 nm.

MASS SPECTROMETRY

Mass spectrometry was performed on a 6530 Accurate - Mass Q-ToF, interfaced to the LC system with a ElectroSpray Ionization source (ESI) operating in negative mode. The employed Q-ToF mounts a Jet Stream thermal gradient focusing system, which dramatically improves detector sensitivity (to femtogram levels of detection) and reduce the noise .
The operating parameters were fixed as follow: Gas temperature 300 ºC; gas flow 8 L/min; nebulizer 55 psi; sheath gas temp 400ºC; sheath gas flow 12 l/min; capilar voltage (-) 3500 V; fragmentor 185 V. Acquisition was done in MS and MS/MS mode, within a mass range of 100-1700 m/z units. For indigoid dyestuffs MS detector worked also in positive mode (Electrospray ESI+) with the same conditions, but with a gas flow of 5 L/min. All the mass spectrometer operating parameters are specified in detail in

Tab. 5.1 - HPLC-W-ToF-MS operating parameters

Stationary phase (column)	Zorbax-C18 SB column (50 x 2.1 mm,1.8 µm)
Mobile phase (solvents)	A: H2O-0,1% HCOOH B: ACN
Flow	0,75 ml/min
Injected volume	1 µl o 2 µl
Temperature	35ºC
Stop time	25
Post time	3
Detector DAD	scan 200 nm to 800 nm
Monitorización DAD	275 nm, 350 nm, 550 nm, 600 nm

Timetable

time	B%
0	10
0,4	10
6,5	22
8	22
14	35
18	95
21	95
25	10

Mass spectrometer model	6530 Accurate-Mass Q-ToF
Polarity	negative
Gas temp	300ºC
Drying	5 l/min
Nebulizer	55 psig
Sheath gas flow	12 l/min
Sheath gas temperature	400ºC
Vcap (-)	3500
Nozzle volt	1000 V
Fragmentor	185V
Skimmer	65V
Oct	750V
Collision energy	35 V
Acquisition mode	scan MS y Auto MS/MS
MS range	100-1700 m/z
MS/MS range	100-1700 m/z
MS and MS/MS scan rate	3 spectra/s

Tab. 5.1 - HPLC-W-ToF-MS operating parameters

DATA PROCESSING

Data acquisition and processing were performed using MassHunter Software (Workstation, Qualitative Analysis Software and PCDL Manager). Data processing involved the extraction of UV-VIS chromatogram (usually at 275, 550 and 600 nm), the integration of the relative peaks, the extraction of the mass-signal chromatograms (EIC - Extracted Ion Chromatogram) with relative MS and MS/MS spectra and the identification of extracted compounds. The first step of interpretation phase was the comparison with a reference database specifically created in IPCE facilities for the organic dyes analysis.

5.1.4 METHOD RELIABILITY ASSESSMENT

A standard reference solution has been used to monitor the method reliability throughout the whole analysis period. The reference mix was composed of 20 common natural dyes chosen to cover the entire chromatogram. To achieve the desiderated mix concentration a proper quantity of each analytical standard has been taken (see Tab. 5.2). The volume have been adjusted to 1,5 ml by adding a 50% v/v methanol /dimethylformamide solution. All the analytical standards and the solvents used were from Sigma Aldrich (St. Louis, MO, USA).

1. Gallic acid	10 µl	11. Flavokermesic acid	30 µl
2. Carminic acid	10 µl	12. Genistein	10 µl
3. Ellagic acid	10 µl	13. Apigenin	10 µl
4. Lawsone	8 µl	14. Kaempferol	10 µl
5. Fisetin	8 µl	15. Alizarin	10 µl
6. Morin	8 µl	16. Purpurin	10 µl
7. Sulfuretin	10 µl	17. Indigotin/Indirubin	60 µl
8. Quercetin	10 µl	18. Dibromoindigotin	30 µl
9. Luteolin	10 µl	19. Emodin	10 µl
10. Kermesic acid	30µl	20. Curcumin I	10 µl

Tab. 5.2 - Amount of the analytical standards composing the reference mix.

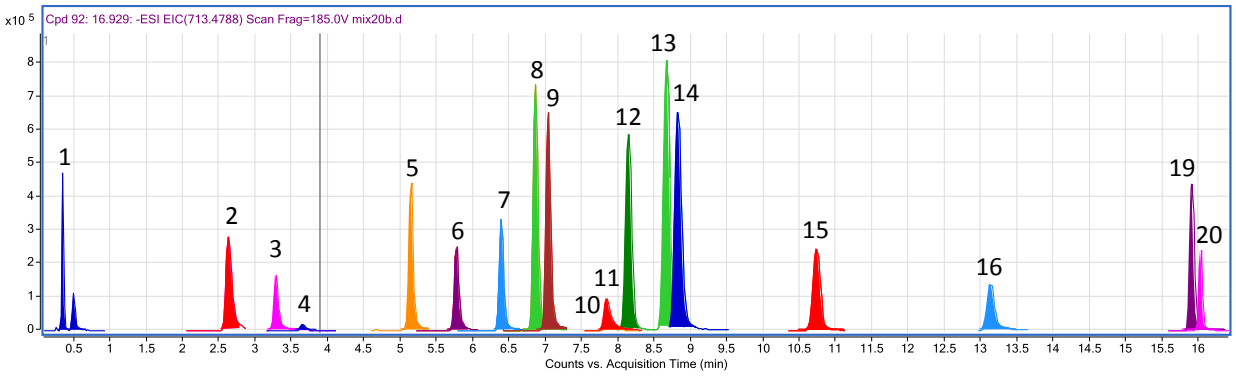


Fig. 5.1 – Extracted Ion Chromatogram (EIC) of the reference mix

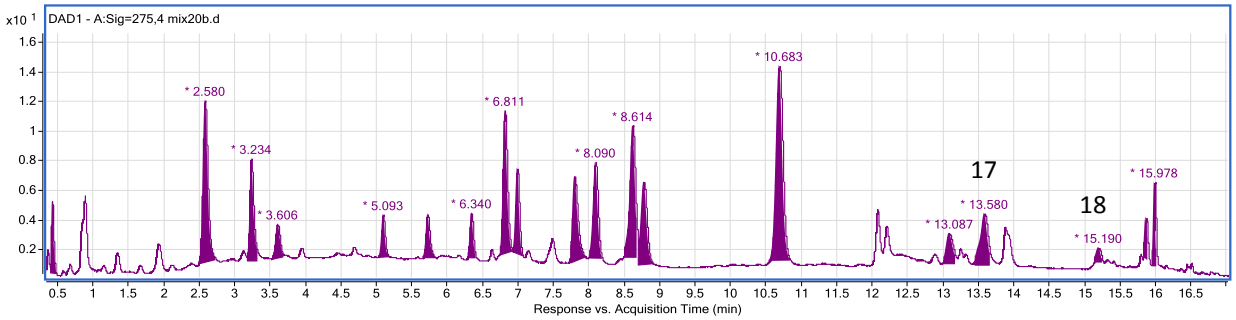
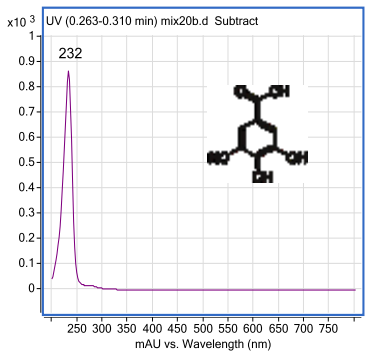
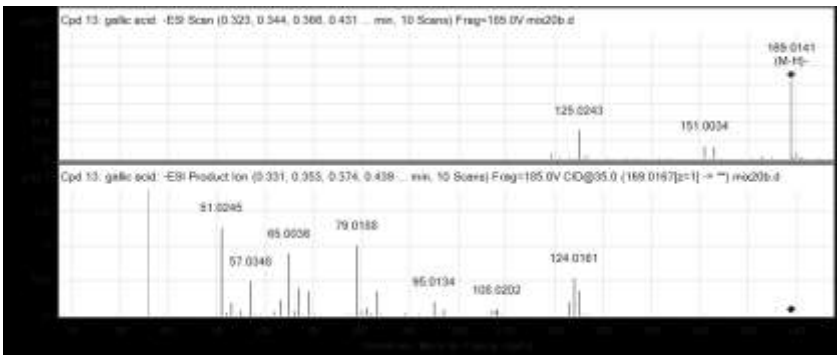


Fig. 5.2 – DAD Chromatogram at 275 nm of the reference mix

RT : 0,35

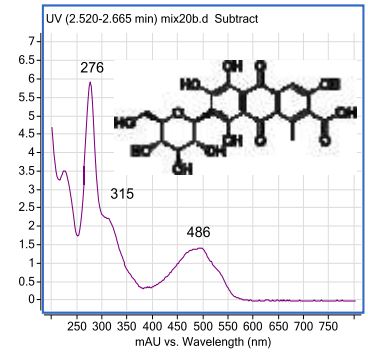
GALLIC ACID Formula: C7H6O5 MW: 170,0219





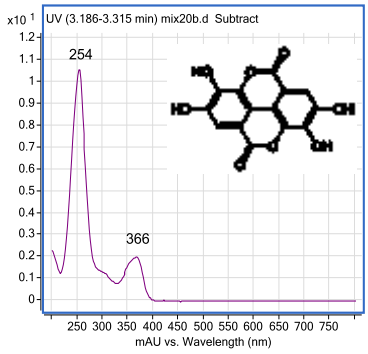
RT: 2,63

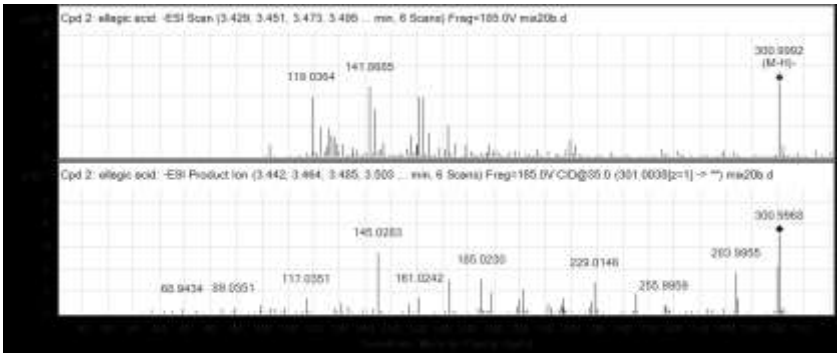
CAMINIC ACID Formula: C22H20O13 MW: 492,0907



RT:3,28

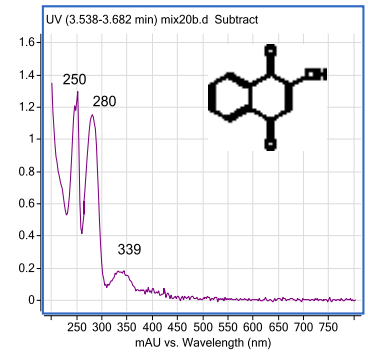
ELLAGIC ACID Formula: C14H6O8 MW: 302,0063

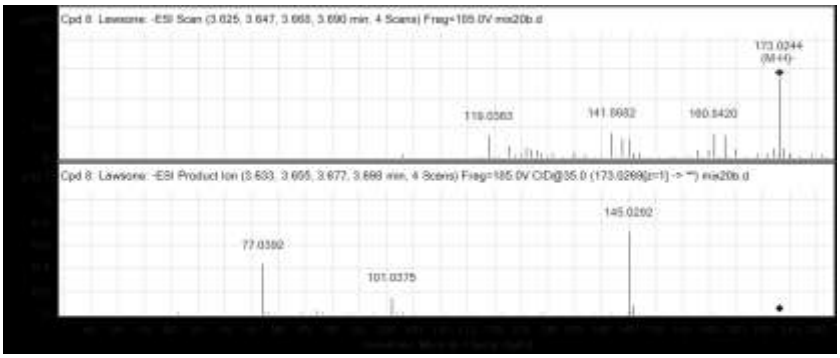




RT: 3,6

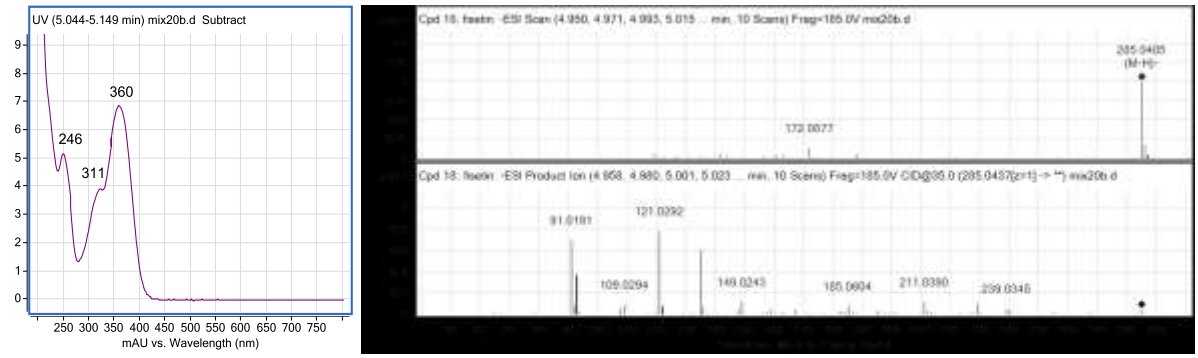
LAWSONE Formula: C10H6O3 MW: 174,0319





RT: 5,14

FISETIN Formula: $C_5H_{10}O_6$ MW: 286,0481



RT: 5,77

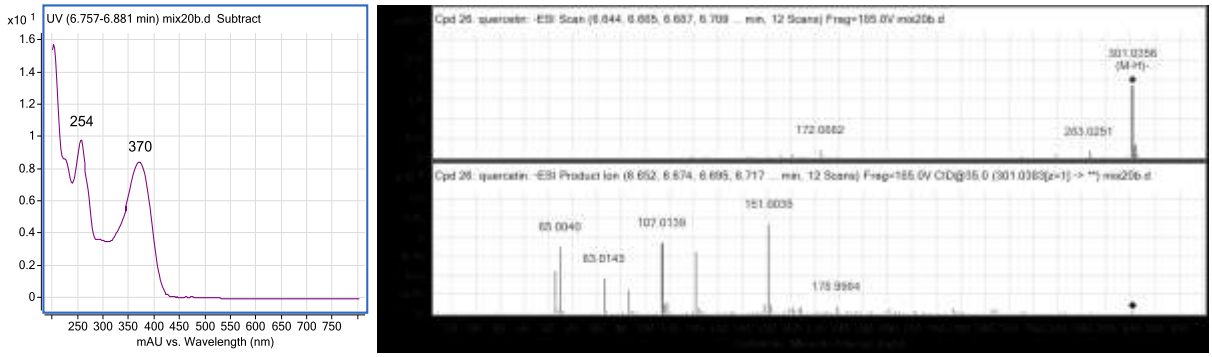
MORIN Formula: $C_{15}H_{10}O_7$ MW: : 302,0426

RT: 6,39

SULFURETIN Formula: $C_{15}H_{10}O_5$ MW: 270,0530

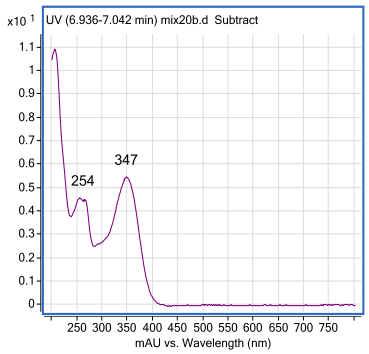
RT: 6,86

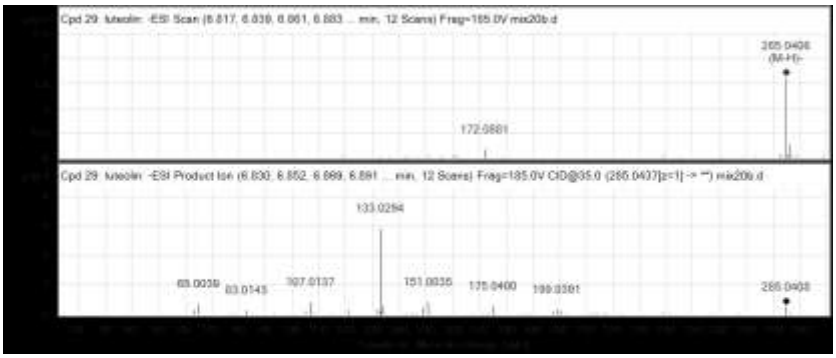
QUERCETIN Formula: $C_{15}H_{10}O_5$ MW: 302,0426



RT: 7,03

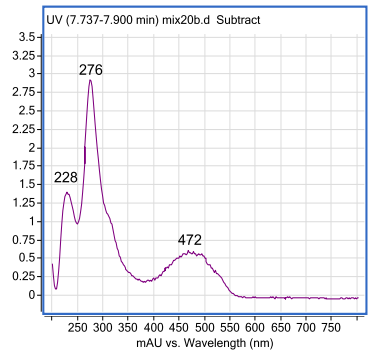
LUTEOLIN Formula: $C_{15}H_{10}O_6$ MW: 286,0477

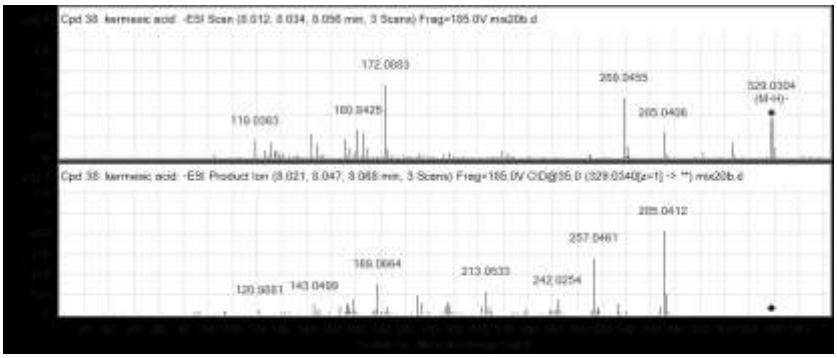




RT: 7,84

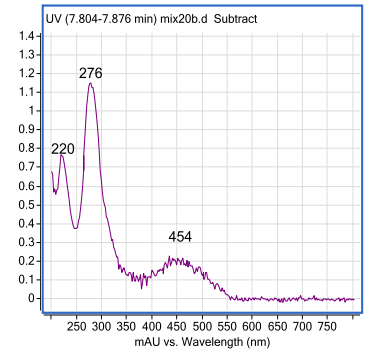
KERMESIC ACID Formula: $C_{16}H_{10}O_8$ MW: 330,0376

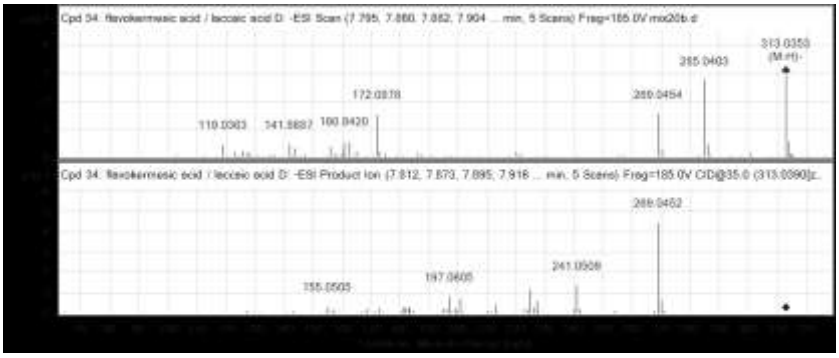




RT: 7,86

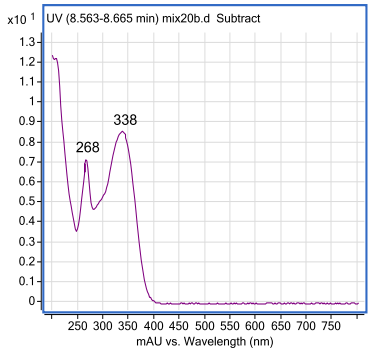
FLAVOKERMESIC ACID Formula: $C_{16}H_{10}O_7$ MW: 314,0426

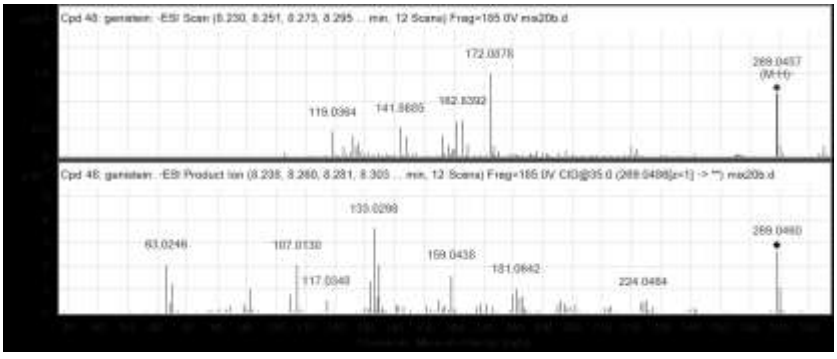




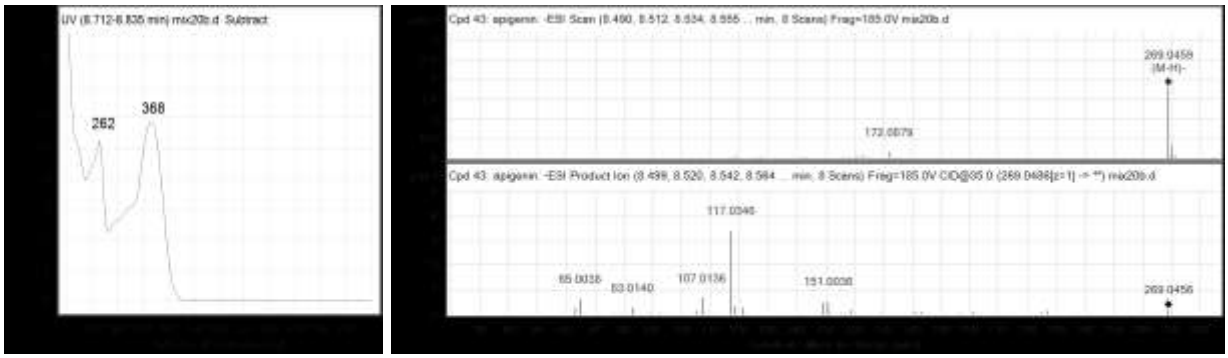
RT: 8,14

GENISTEIN Formula: $C_{15}H_{10}O_5$ MW: 270,0528

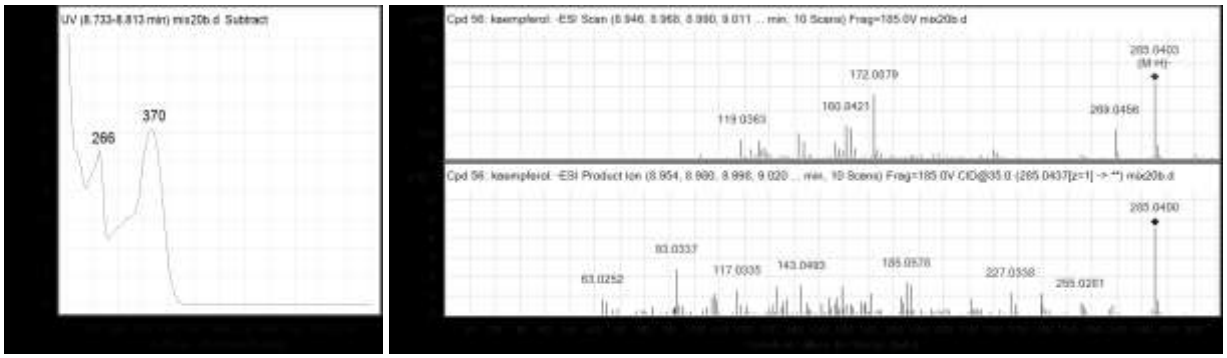




RT: 8,66
APIGENIN Formula: $C_{15}H_{10}O_5$ MW: 270, 0528

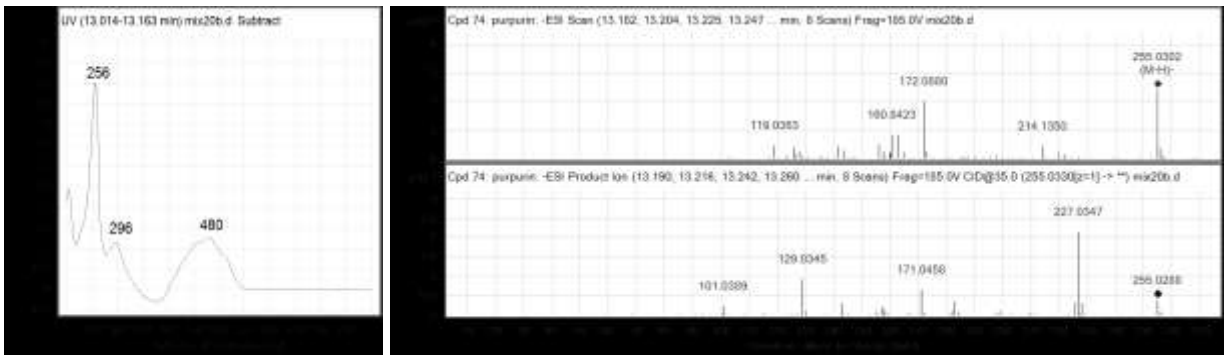


RT: 8,82
KAEMPFEROL Formula: $C_{15}H_{10}O_6$ MW: 286,0477

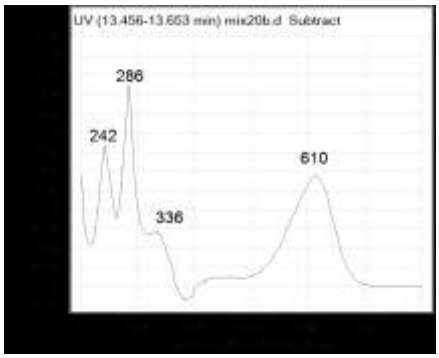


RT: 10,73
ALLIZARIN Formula: $C_{14}H_8O_4$ MW: 240,04226

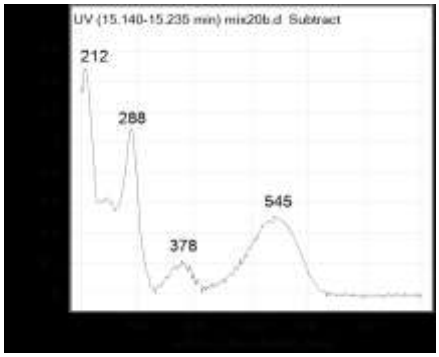
RT: 10,73
PURPURIN Formula: $C_{14}H_8O_5$ MW: 256,0371



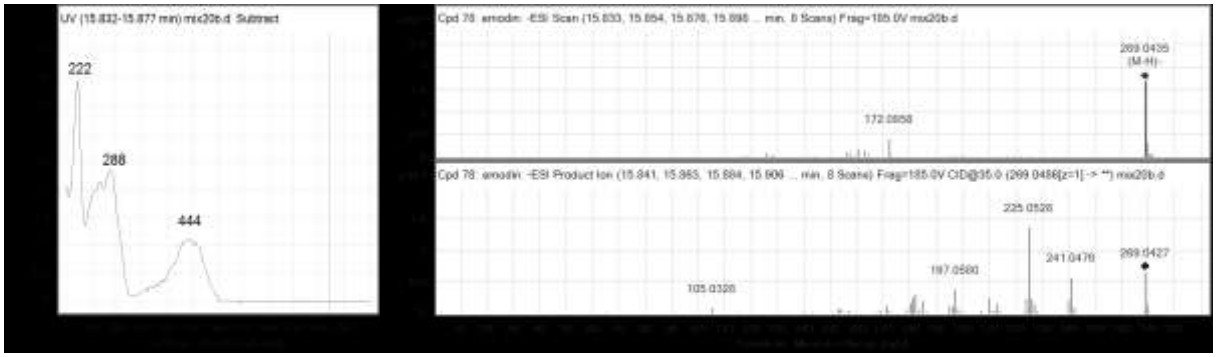
RT: 13,58
INDIGOTIN Formula: $C_{16}H_{10}N_2O_2$ MW: 262,07423



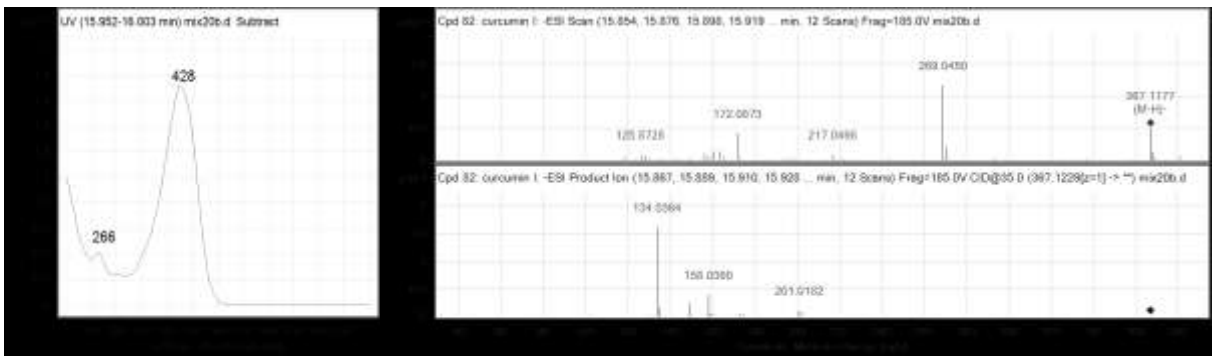
RT: 13,58
INDIRUBIN Formula: $C_{16}H_{10}N_2O_2$ MW: 262,07423



RT: 15,90
EMODIN Formula: $C_{15}H_{10}O_5$ MW: 270,07356



RT: 16,02
CURCUMIN I Formula: $C_{21}H_{20}O_6$ MW: 368,12599



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6. ANTHRAQUINONIC DYES

Cochineal 

Madder 

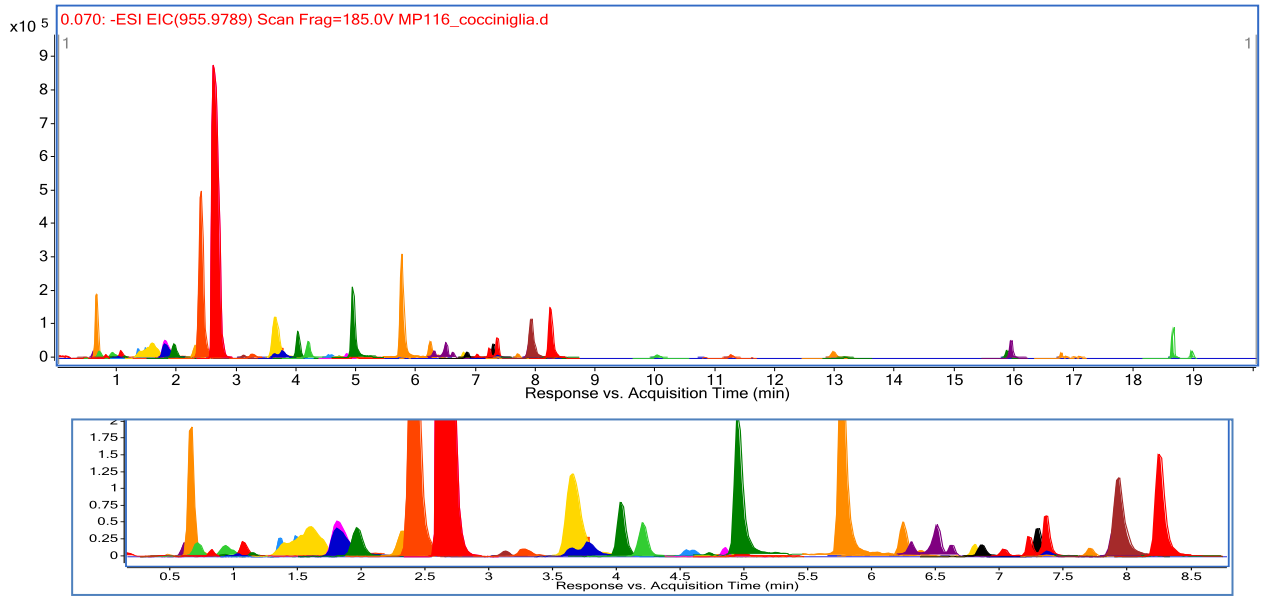
Lac dye 

6.1 COCHINEAL (COCCUS CACTI)

MB 110	MB 111	MB 137A	MB 137B	MB 139	MP115 A
S 2050-R40B	S 3020-R10B	S 5040-R30B	S 3050-R30B	S 2040-R40B	S 5040-R10B
cochineal clippings, lye, alum	cochineal, lye, quicklime, alum	cochineal, lye, alum, rock salt	cochineal, alum, lye rock salt, calcite	cochineal clippings, lye, alum	cochineal, alum, lye, fennel seeds
hot extraction with lye	hot extraction with lye and quicklime	hot extraction with lye	hot extraction with lye	cold extraction with lye	hot extraction with water

MP 115B	MP 116	COCMEX	COCPER	COC1	COC2
S 6030-R30B	S 3055-R40B				
cochineal, alum, lye, fennel seeds	cochineal, lye, alum	cochineal unknown recipe	cochineal unknown recipe	cochineal	cochineal
hot extraction with water	cold extraction with lye				

Among all the recurrent compounds identified in the analyzed lakes, twelve of them have been selected as universal markers of cochineal lakes due to their presence in (almost) all the samples. Hereinafter is presented an exemplificative chromatogram with labelled these compounds.



The first significant compound elutes at 0.7 min. Since it was an unknown compound, it has been identified with the molecular formula $C_{20}H_{20}O_{12}$ and labelled "unk 451 cochineal"(1). It presents the typical UV-VIS spectrum of tannins with a maximum of absorptions at 278 and 306 nm. From the study of fragmentation pattern it has not been possible to relate it with any of the known structures.

At 1.46 min elutes the compound "unk 203 cochineal" identified by the molecular formula $C_{11}H_8O_4$ (2). Despite the peak height indicates a very low concentration of such compound in the analysed extract, in the UV-VIS spectra it has been possible to recognize the typical absorption pattern of anthraquinonic compounds (with max at 272, 302, 480 nm). The study of fragmentation pattern in the MS/MS spectra seems to confirm this evidence since it reveals the loss of –CHO fragments (-28 Amu) typical of this family of compounds.

At minute 2.27 elutes a C-glucoside of flavokermesic acid (3). According to its MS/MS fragmentation and its UV-VIS spectrum, it has been identified as dcII, one of the three compounds recognise as natural constituents of American cochineal by Wouters and Verhecken. The authors did not achieve a chemical identification of the compounds labelled them with the common abbreviation "dc" (from Dactylopius Coccus).

Carminic acid, and its isomers dcIV and dcVII elute at minute 2.50, 4.1 and 5.6 respectively. They are impossible to distinguish by means of diode array detection because their UV-VIS spectra present the same absorptions bands at 224, 276, 314 and 484 nm. The fragmentation pattern is almost identical suggesting that probably they differ only in the stereochemistry of the sugar moiety. Their MS/MS spectra presents a initial loss of 44 amu due to decarboxylation of the carboxylic acid (leading to the fragment at m/z 447) followed by the loss of 120 Amu (90 + 30) related to the loss of the sugar residue. (m/z fragments: 447, 357, 327).

A new anthraquinonic compound characterized by m/z 507.0772 and molecular formula $C_{22}H_{20}O_{14}$, has been detected at minute 2,33. It presents a fragmentation pattern similar to that of carminic acid with an initial loss of a carboxylic group (-44 amu), living the fragment 463, and consecutive loss of a sugar moiety (-120 amu) giving the fragments 373 (-90) and 343 (-30). According to the UV-VIS absorption at 242, 272, 316, 504 nm it presents a reddish-purple hue.

At minute 3.76 elutes another coloured compound that have been labelled "unk 475 cochineal". It presents absorption bands at 220, 284, 428 nm as usual in anthraquinones. Its MS/MS spectra revealed an initial decarboxylation followed by loss of a hexose sugar (- 162 amu). The fragmentation pattern is typical of O - Glycosides. Flavokermesic acid exits at 7.62 minutes. Its MS/MS spectrum presents fragments 269, 241, 225, 197 that indicates the loss the carboxylic group (-44 Amu). Table XXX reports the list of all compound classified as "recurrent" in cochineal lakes. Samples analysis shown that, in addition to these compounds, each sample presented an own.

RT	COMPOUND NAME	FORMULA	MASS	M/Z	MS/MS FRAGMENTS
0,516	unk 253 cochineal	C11 H10 O7	254,0414	253,0341	**253**, 201, 133, 106
0,587	mono caffeoylquinic acid	C16 H18 O9	354,1058	353,0985	**353**, 191, 179, 135, 127, 93, 85
0,695	unk 451 cochineal	C20 H20 O12	452,0956	451,0884	**451**, 287, 269, 259, 241, 225, 215, 203, 197, 175, 131
0,728	monohidroxy benzoic acid	C7 H6 O3	138,032	137,0247	**137**, 106, 96, 93, 79, 71, 65
0,728	unk 571 cochineal	C22 H20 O16 S	572,0469	571,0396	**571**, 509, 445, 388, 297, 269
0,732	unk 447 cochineal	C25 H20 O8	448,1119	447,1046	**447**, 403, 313, 283, 254, 241, 210, 198
0,775	unk 463 I cochineal	C21 H20 O12	464,0951	463,0878	**463**, 445, 343, 325, 299, 281, 269, 257, 253, 225, 215, 189
0,895	unk 177 cochineal	C9 H6 O4	178,0263	177,0191	**177**, 154, 140, 115, 105, 99
0,952	unk 345 cochineal	C17 H18 N2 O6	346,1157	345,1084	**345**, 223, 211, 193, 167
1,032	unk 439 cochineal	C19 H20 O12	440,0957	439,0884	**439**, 231, 191, 135, 119, 107, 97
1,073	unk 175 cochineal	C10 H8 O3	176,0471	175,0398	**175**, 14, 131, 116, 105, 103
1,147	hydroxybenzaldehyde	C7 H6 O2	122,0373	119,0363	**119**, 92, 63
1,303	unk 445 cochineal	C21 H18 O11	446,0838	445,0766	**445**, 343, 325, 299, 281, 269, 257, 253, 225, 215, 189
1,352	unk 669 cochineal	C28 H30 O19	670,1383	669,131	**669**, 625, 447, 429, 357, 327, 299, 195, 129, 75
1,412	unk 281 Cochineal	C12 H10 O8	282,0379	281,0307	**281**, 177, 133, 106, 105
1,414	unk 653 cochineal	C28 H30 O18	654,1437	653,1364	**653**, 635, 609, 591, 429, 369, 357, 339, 327, 309, 299, 285
1,461	unk 203 cochineal	C11 H8 O4	204,0421	203,0349	**203**, 175, 146, 131, 107, 105, 103
1,561	unk 379 cochineal	C16 H32 N2 O6 S	380,1975	379,1902	379, 347, 293, 179, 96, 79
1,84	unk 571 cochineal	C22 H20 O16 S	572,0467	571,0394	**571**,491, 473, 447, 383, 357, 327, 299
1,844	carminic acid isomer	C22 H20 O13	492,09	491,0827	**491**, 473, 383, 357, 299, 281, 269, 227
1,873	unk 263 cochineal	C21 H20 O14 S	528,058	263,0217	**263**, 241, 96, 79
1,953	unk 423 Cochineal	C19 H20 O11	424,1006	423,0933	**423**, 259, 217, 201, 175, 131
2,187	unk 381cochineal	C16 H34 N2 O6 S	382,2134	381,2062	**381**, 363, 348, 96, 79
2,276	dcII C- glucoside of flavokermesic acid	C22 H20 O12	476,0942	475,0870	**475**, 353, 341, 323, 311, 295, 282, 269, 254
2,33	unk 507 cochineal	C22 H20 O14	508,0845	507,0772	**507**, 463, 373, 355, 343, 330, 315, 301, 286
2,495	carminic acid	C22 H20 O13	492,0905	491,0832	**491**, 403, 391, 379, 355, 351, 337, 325, 309, 299, 281
3,127	unk 537 cochineal	C23 H22 O15	538,0945	537,0872	**537**, 269, 241, 103
3,643	quercetin-3-O-glucuronide	C21 H18 O13	478,0744	477,0671	**477**, 301, 283, 273, 255, 245, 178, 11, 121
3,701	unk 178 cochineal	C9 H6 O4	178,0267	177,0194	**177**, 105
3,764	unk 475 cochineal	C22H20O12	476,0956	475,0883	**475**, 431, 268, 240
4,034	unk 490 cochineal	C23H22O8S2	490,0963	489,0890	**489**, 447, 399, 369 , 357, 341 , 327, 309, 297, 284, 269, 255, 241, 149, 78
4,154	dcIV- carminic acid isomer	C22 H20 O13	492,0899	491,0832	**491**, 447, 369, 357, 339, 327, 311, 299, 285, 270

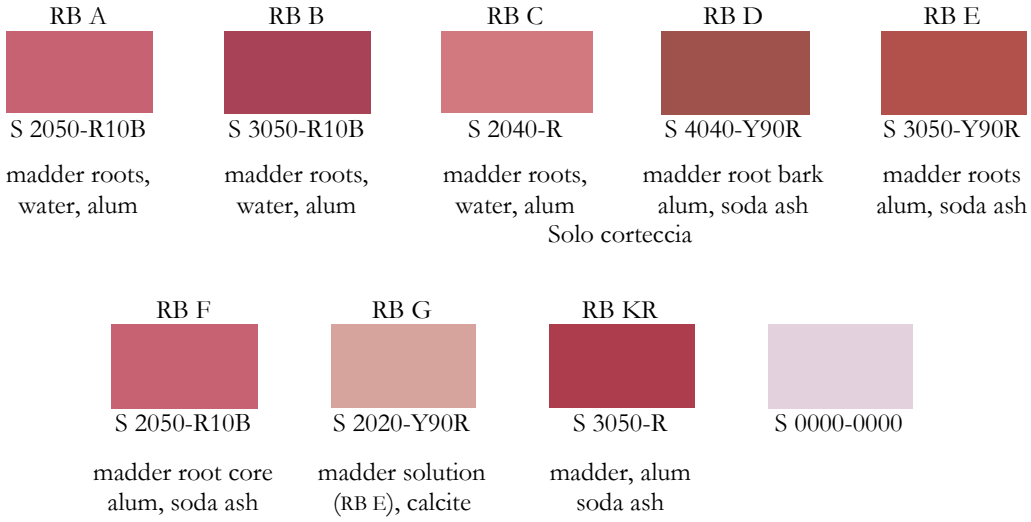
4,619	unk 499 cochineal	C38 H12 O2	500,0861	499,0788	499, 241, 137, 96, 78
4,625	unk2- 520 cochineal	C27 H20 O11	520,084	519,0767	**519**, 475, 393, 369, 357,339, 327, 311, 299, 285
4,877	unk 345 cochineal	C16 H10 O9	346,0315	345,0243	**345**, 301, 273, 255, 227, 201, 173, 145
4,941	unk 447 cochineal	C21 H20 O11	448,0997	447,0925	**447**, 424, 357, 298, 270, 210
4,997	unk 463 cochineal	C21 H20 O12	464,095	463,0878	**463**, 445, 385, 373, 355, 343, 327, 314, 300, 286, 271
5,117	unk 596 II cochineal	C28 H23 N O14	597,1112	596,1039	**596**, 552, 429, 411, 339, 321, 313, 299, 285
5,345	unk 625 cochineal	C34H26O12	626,143	625,1357	**625**, 447, 429, 369, 339, 326, 309, 297, 285
5,394	unk 475 cochineal	C22 H20 O12	476,0949	475,0876	475, 341, 323, 311, 295, 282, 269, 254
5,629	dcVII- carminic acid isomer	C22 H20 O13	492,1041	491,0968	**491**, 447, 369, 357, 339, 327, 311, 299, 285, 270
6,268	unk 473 cochineal	C22 H18 O12	474,0794	473,0721	**473**, 429, 369, 351, 339, 321, 311, 298, 285, 270
6,5	unk 719 cochineal	C34 H40 O17	720,2262	719,2189	**719**, 675, 429, 339, 309
6,594	unk 775 cochineal	C38 H52 N2 O15	776,337	775,3298	**775**, 473
6,615	unk 648 cochineal	C32 H27 N O14	649,1438	648,1365	**648**, 429, 369, 339, 326, 309, 297
6,782	unk 401 Cochineal	C19 H14 O10	402,0585	401,0512	**401**, 357, 339, 321, 311, 299, 284, 270, 254, 241, 225, 183
7,089	unk 561 cochineal	C30 H26 O11	562,1464	561,1391	**561**, 429, 339, 309
7,315	unk 773 cochineal	C38 H50 N2 O15	774,3205	773,3132	**773**, 653, 473
7,417	kermesic acid	C16 H10 O8	330,0372	329,0299	**329**, 285, 257, 242, 229, 213, 201, 185, 169, 157, 143, 107
7,62	flavokermesic acid / laccaic acid D	C16 H10 O7	314,0424	313,0351	**313**, 269, 241, 226, 213, 197, 169, 155, 127
7,713	unk 431 cochineal	C21 H20 O10	432,1046	431,0974	**431**, 268, 240
8,191	unk 597 cochineal	C32 H22 O12	598,1103	597,103	**597**, 298, 254, 226
8,352	unk 612 cochineal	C29 H24 O15	612,111	611,1037	**611**, 429, 309, 137
8,844	unk 401 cochineal	C23 H14 O7	402,0717	401,0644	**401**, 397, 313, 298, 285, 270, 241, 226, 197
9,064	unk 611 cochineal	C34 H28 O7 S2	612,1273	611,1201	**611**, 429, 309, 137
9,645	unk 431 cochineal	C21 H20 O10	432,1056	431,0983	**431**, 282, 269, 225
10,021	unk 415 cochineal	C21 H20 O9	416,1099	415,1026	**415**, 266, 253, 225, 209
11,184	unk 641 cochineal	C38 H46 N2 O3 S2	642,2948	641,2875	**641**, 597, 567, 356, 326, 297
11,743	unk 603 cochineal	C33 H32 O11	604,1955	603,1882	**603**, 559, 429, 339, 309
11,744	unk 372 cochineal	C17 H11 N O9	373,0428	372,0355	**272**, 310, 296, 282, 200, 65
12,221	unk 270-2 kermes/cochineal	C15 H10 O5	270,0544	269,0471	**269**, 254, 241, 225, 213, 197, 182, 169, 155, 143, 127
15,811	Emodin	C15 H10 O5	270,0527	269,0454	**269**, 241, 225, 210, 197, 181, 171
15,881	unk 253 cochineal	C15 H10 O4	254,0579	253,0506	**253**, 225, 209, 194, 181, 159, 85, 59
16,645	unk 595 cochineal	C33 H56 O9	596,3913	595,384	**595**, 315, 279, 241, 152, 78

RT	COMPOUND NAME	MOLECULAR FORMULA	MB 110	MB 111	MP 115	MP 115B	MP 116	MB 137A	MB 137B	MP 139
0,516	unk 253 cochineal	C11 H10 O7		■	■	■			■	■
0,568	unk 209 Cochineal	C10 H10 O5		■						■
0,587	mono caffeoylquinic acid	C16 H18 O9			■	■				
0,695	unk 451 cochineal	C20 H20 O12	■	■	■	■	■	■	■	■
0,728	monohidroxy benzoic acid	C7 H6 O3		■			■			
0,728	unk 571 cochineal	C22 H20 O16 S		■	■	■	■			■
0,732	unk 447 cochineal	C25 H20 O8			■			■	■	
0,775	unk 463 I cochineal	C21 H20 O12			■	■		■		
0,837	unk 219 Cochineal	C11 H8 O5		■	■		■	■		
0,895	unk 177 cochineal	C9 H6 O4	■	■						■
0,952	unk 345 cochineal	C17 H18 N2 O6			■	■				
0,99	unk 251 cochineal	C23 H24 N2 O14						■	■	
1,032	unk 439 cochineal	C19 H20 O12		■	■	■				■
1,073	unk 175 cochineal	C10 H8 O3				■				■
1,147	hydroxybenzaldehyde	C7 H6 O2					■			
1,303	unk 445 cochineal	C21 H18 O11			■	■				
1,352	unk 669 cochineal	C28 H30 O19			■	■		■		
1,412	unk 281 Cochineal	C12 H10 O8		■		■	■			■
1,414	unk 653 cochineal	C28 H30 O18			■	■		■		
1,461	unk 203 cochineal	C11 H8 O4	■	■	■	■	■	■	■	■
1,561	unk 379 cochineal	C16 H32 N2 O6 S		■		■	■	■		
1,771	unk 475 cochineal	C29 H16 O7			■	■				
1,84	unk 571 cochineal	C22 H20 O16 S	■	■			■			
1,844	carminic acid isomer	C22 H20 O13	■				■			
1,873	unk 263 cochineal	C21 H20 O14 S	■	■			■			
1,953	unk 423 Cochineal	C19 H20 O11		■		■	■			■
2,118		C21 H18 O11		■	■	■				
2,187	unk 381cochineal	C16 H34 N2 O6 S				■		■		
2,276	dcII C- glucoside of f.a.	C22 H20 O12	■	■	■	■	■	■	■	■
2,33	unk 507 cochineal	C22 H20 O14		■	■	■	■	■	■	■
2,495	carminic acid	C22 H20 O13	■	■	■	■	■	■	■	■
2,716	unk 447 cochineal	C21 H20 O11		■				■	■	
3,127	unk 537 cochineal	C23 H22 O15				■	■			
3,643	quercetin-3-O-glucuronide	C21 H18 O13			■	■				
3,701	unk 178 cochineal	C9 H6 O4	■	■			■		■	
3,805	unk 263 cochineal	C21 H20 O14 S	■	■			■			
4,016	unk 475 cochineal	C22 H20 O12		■	■	■	■	■	■	■
4,034	unk 490 cochineal	C23H22O8S2			■	■			■	
4,039	dcIV- carminic acid isomer	C22 H20 O13	■	■	■	■	■	■	■	■
4,154	carminic acid isomer	C22 H20 O13	■		■		■		■	■
4,327	unk 563 cochineal	C31 H26 O16			■	■				
4,469	unk 596 cochineal	C28 H23 N O14				■		■		
4,619	unk 499 cochineal	C38 H12 O2			■	■				
4,625	unk2- 520 cochineal	C27 H20 O11			■	■	■		■	
4,877	unk 345 cochineal	C16 H10 O9			■	■				■
4,941	unk 447 cochineal	C21 H20 O11			■	■	■			
4,997	unk 463 cochineal	C21 H20 O12					■		■	
5,117	unk 596 II cochineal	C28 H23 N O14				■		■		

5,345	unk 625 cochineal	C35 H30 O7 S2			■	■				
5,394	flavokermesic acid isomer	C22 H20 O12		■	■	■				
5,629	dcVII- carminic acid isomer	C22 H20 O13	■	■	■	■	■	■	■	■
6,14	unk 539 cochineal	C30 H20 O10	■			■				
6,263	unk 611 cochineal	C33 H24 O12			■	■		■	■	
6,268	unk 473 cochineal	C22 H18 O12		■	■		■	■	■	
6,381	unk 639 cochineal	C31 H28 O15			■	■				
6,5	unk 719 cochineal	C34 H40 O17			■	■	■			
6,594	unk 775 cochineal	C38 H52 N2 O15			■	■				
6,615	unk 648 cochineal	C32 H27 N O14			■	■				
6,782	unk 401 Cochineal	C19 H14 O10					■			■
7,089	unk 561 cochineal	C30 H26 O11								
7,315	unk 773 cochineal	C38 H50 N2 O15			■	■			■	
7,417	kermesic acid	C16 H10 O8			■					
7,62	flavokermesic acid	C16 H10 O7	■	■	■	■	■	■	■	■
7,637	unk 270 kermes	C15 H10 O5	■	■	■	■	■	■	■	■
7,713	unk 431 cochineal	C21 H20 O10			■	■	■			
7,951	unk 303 cochineal	C16 H32 O5		■						■
8,191	unk 597 cochineal	C32 H22 O12	■	■	■	■	■	■	■	■
8,281	unk 254 cochineal	C13 H9 N3 O3	■				■			
8,352	unk 612 cochineal	C29 H24 O15			■	■				
8,844	unk 401 cochineal	C23 H14 O7			■	■				
9,043	unk 215 cochineal	C11 H20 O4		■						■
9,064	unk 611 cochineal	C34 H28 O7 S2								
9,141	unk 245 cochineal	C12 H22 O5		■						■
9,645	unk 431 cochineal	C21 H20 O10			■	■				
10,021	unk 415 cochineal	C21 H20 O9			■	■	■			■
11,184	unk 641 cochineal	C38 H46 N2 O3 S2								
11,743	unk 603 cochineal	C33 H32 O11			■	■				
11,744	unk 372 cochineal	C17 H11 N O9				■				■
11,885	unk 229 cochineal	C12 H22 O4		■						■
12,221	unk 270-2 cochineal	C15 H10 O5	■	■	■	■	■			■
13,901	unk 341 cochineal	C18 H30 O6		■						■
15,001	unk 608 cochineal	C31 H28 O13								
15,811	Emodin	C15 H10 O5	■	■	■	■	■			■
15,881	unk 253 cochineal	C15 H10 O4	■	■	■		■			
16,645	unk 595 cochineal	C33 H56 O9			■					■
17,928	unk 311 cochineal	C21 H28 O2						■		■

6.1 MADDER

Nine Madder Lake Samples have been prepared and analyzed within the research project.



The primverosides of alizarin, purpurin and rubiadin elute at minute 5.6, 5.7 and 8.3 respectively. They have been easily identified in the respective MS/MS spectra by observation of the characteristic neutral loss of 295 amu. A comparable loss of an hexose sugar (-162 amu) allowed the identification of lucidin, purpurin, alizarin glucoside coeluting at minute 6.

At minute 8,3 an unknown compound characterized by m/z 593,1515 elutes. According to its exact mass and to the isotopic distribution, the molecular formula $C_{27}H_{30}O_{15}$ has been attributed to the compound. The study of fragmentation pattern revealed a neutral loss of 340 amu giving a m/z fragment of 253. This loss can be attributed to a ketolactose or ketosucrose moiety linked to a rubiadin molecule. At minute 8,7 elutes the anthraquinone anthragallol ($C_{14}H_8O_5$) identified by its fragmentation pattern and its UV-VIS spectrum with absorbtion at 248, 286, and 420 nm.

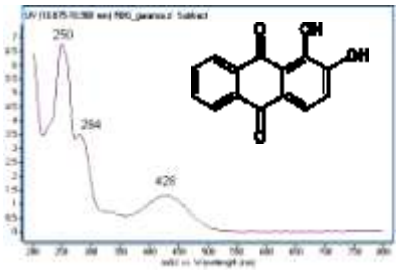
At minute 8,3 elute a compound having m/z 495.0675 (unk 495 madder). Its MS/MS spectra shows a neutral loss of 242 Amu corresponding to the combined loss of an hexose sugar (-162 amu) and sulfur trioxide (80 amu) in collision-induced dissociation. This loss is confirmed by the molecular formula $C_{21}H_{20}O_{12}S$, attributed to the compound on the basis of the exact mass and the isotopic distribution.

Another unknown compound elutes at minute 9,3. It is characterized by m/z 415,1031 and molecular formula $C_{21}H_{20}O_9$. In this case the attribution to a specific class by means of its UV-VIS absorption bands has not been possible due to the presence of a recurrent contaminant that elutes at the same retention time. However, the fragmentation pattern with loss of 162 amu to give the fragment m/z 253, clearly indicates that it is a rubiadin hexoside.

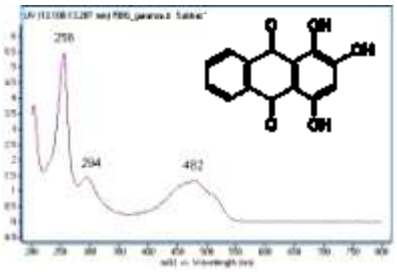
At 10,23 elutes another anthraquinon derivative. The loss of a 198 amu neutral fragment could be explained with the loss of a galactose moiety (-180 amu) plus a water molecule (-18 amu). The presence of the fragment m/z 253, and the initial loss of 44 amu ascribable to decarboxylation process, induces to identify the aglycon ($C_{16}H_{10}O_6$) as a rubiadin isomer containing a carboxylic group in its structure (e.g. 2,5-Dihydroxy-6-methylanthraquinone-1-carboxylic acid). The same fragmentation pattern is observed in a compound eluting at 10,98 min suggesting that it could be an isomer with changes in the position of the substituent groups.

Another unknown compound identified by molecular formula $C_{16}H_{10}O_8$ (unk 320 madder) elutes at minutes 10,8. It has not been possible to gather information from the UV-VIS chromatogram because it co-elutes with alizarin. However, its fragmentation pattern suggests it is an anthraquinonic compound. MS/MS spectrum presents m/z 328, 300, 272, 244, 216 fragments given by four consecutive losses of CO (-28 amu) highly characteristic of dihydroxyanthraquinones.

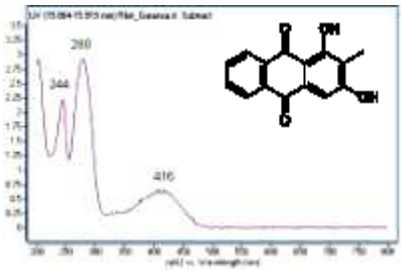
The three main aglycones of mader lake, alizarin ($C_{14}H_8O_4$), purpurin ($C_{14}H_8O_5$) and rubiadin ($C_{15}H_{10}O_4$), elute at 10.8, 13.4 and 15.9 minutes respectively. The respective UV-VIS spectra are shown in figure XXX.



alizarin



purpurin



rubiadin

At minute 12,1 a compound with mass 267,0298 have been detected in the MS chromatogram. It has been identified with the label "unk 267 madder". It seems not to have a correspondence in the DAD Chromatogram suggesting it is a uncoloured substance or that its concentration is lower to de detection limit of the Diode Array Detector. The molecular formula $C_{15}H_8O_5$ has been attributed to the compound. The fragmentation pattern shows an initial loss of CO_2 (-44 amu) followed by loss of a CO unit (-28) amu. An unknown compound with molecular formula $C_8H_{10}O_7S_4$ co-elutes with purpurin at minute 13.2.

The last significant compound detected in the chromatogram is a reddish compound with formula $C_{29}H_{16}O_9$. The study of fragmentation revealed the loss of a rubiadin molecule (-253) to give m/z 255,0287 corresponding to a purpurin molecule. Even the UV-VIS spectra confirm the hypothesis of a purpurin-rubiadin adduct since the max absorbance fall at an intermediate value (452 nm) between those observed in the two compounds.
Table xxx resumes the recurrent compounds identified in madder lakes from Rubia tinctoria.

RT	COMPOUND NAME	FORMULA	MASS	M/Z	MS/MS FRAGMENTS
0,62	unk 243 madder	C13 H8 O5	244,0373	243,03	**243**, 199, 187, 173, 145, 129, 101
0,94	benzoic acid	C7 H6 O2	122,0366	121,0293	**121**, 110, 77
5,66	alizarin primeveroside	C25 H26 O13	534,137	533,1297	**533**, 239
5,70	lucidin primveroside	C26 H28 O14	564,1483	563,1411	**563**, 282, 269, 265, 251, 237
5,71	purpurin primeveroside	C25 H26 O14	550,132	549,1247	**549**, 255
6,04	lucidin glucoside	C21 H20 O10	432,1052	431,0979	**431**, 269, 251, 237, 209
6,07	purpurin glucoside	C20 H18 O10	418,0883	417,081	**417**, 255, 96
6,09	alizarin glucoside	C20 H18 O9	402,0948	401,0875	**401**, 239, 211
7,56	unk 415 madder	C21 H20 O9	416,1107	415,1035	**415**, 252, 237, 113, 101, 89, 71, 59
7,78	unk 283 madder	C16 H12 O5	284,0683	283,061	**283**, 253, 239, 210
8,33	rubiadin primveroside	C26 H28 O13	548,1527	547,1455	**547**, 253
8,34	unk 495 madder	C21 H20 O12 S	496,0675	495,0602	**495**, 451, 253, 241, 138, 96
8,34	unk 593 madder	C27 H30 O15	594,1588	593,1515	**593**, 253
8,63	unk 431 madder	C21 H20 O10	432,1054	431,0981	431, 268, 253, 240, 223
8,69	anthragallol	C14 H8 O5	256,0375	255,0302	**255**, 237, 227, 210, 198, 181, 171, 153, 143, 125, 115,101
9,30	rubiadin glucoside	C21 H20 O9	416,1103	415,1031	**415**, 266, 253, 225, 209
10,10	3-hydroxy anthraquinone	C14 H8 O3	270,0534	269,0461	**269**, 251, 223, 211, 195, 179, 167
10,24	unk galactoside	C17 H20 O17	496,0674	495,0601	**495**, 451, 253, 241, 152, 96
10,48	alizarine 1-methyl ether ?	C15 H10 O4	254,0572	253,05	253, 239, 225, 223, 209, 195, 181, 169, 138, 110, 83
10,83	unk 329 madder	C16 H10 O8	329,9384	328,9311	**328**, 300, 272, 244, 216, 210
10,84	unk 501 madder	C30H14O10	502,0666	501,0593	501, 239
10,98	unk galactoside	C17 H20 O17	496,0674	495,0601	**495**, 451, 253, 241, 96
10,9	munjistin	C15 H8 O6	284,0319	283,0246	**283**, 239, 211, 195, 167, 1
11,16	alizarin	C14 H8 O4	240,0423	239,035	**239**, 210, 195, 183, 167, 155, 127, 101
13,18	purpurin	C14 H8 O5	256,0375	255,0302	**255**, 227, 183, 171, 157, 143, 129, 101
12,16	unk 267 madder	C15 H8 O5	268,0371	267,0298	**267**, 223, 195
14,29	unk 283 madder	C16 H12 O5	284,0685	283,0613	**283**, 251, 223, 195, 179, 167
15,90	rubiadin	C15 H10 O4	254,0583	253,051	**253**, 225, 209, 181
16,13	unk 282 madder	C15 H9 N O5	283,0484	282,0411	**282**, 264, 236, 224, 195, 65
16,19	unk 252 madder	C13 H7 N3 O3	253,05	252,0427	**252**, 223, 195
16,77	unk 425 madder	C25 H14 O7	426,0739	425,0666	**425**, 251, 239, 211, 195, 173, 145
16,84	unk 275 madder	C18 H12 O3	276,0783	275,071	276, **275**, 273, 232, 231, 230
17,66	unk 491 madder	C29 H16 O8	492,0844	491,0771	**491**, 251, 239
17,84	unk 507 madder	C29 H16 O9	508,0786	507,0713	**507**, 255, 251, 239

9. OTHER DYES

Alkanna 

Gamboge 

Indian yellow 

Rathany 

Saffron 

9.1 ALKANNA (ALKANNA TINCTORIA)

Tab. 9.1 – Main compounds detected in alkanna lakes

RT	NAME	FORMULA	MASS	M/Z	BASE PEAK
13,33	unk alkanna	C9 H5 N3 O5	235,0243	234,017	177,0188
3,76	unk alkanna	C27 H22 O6 S	474,1149	473,1076	311,0553
3,03	unk alkanna	C16 H10 O6	298,0477	297,0404	209,061
16,87	unk alkanna	C16 H30 O4	286,2142	285,2069	223,2058
13,36	unk alkanna	C30 H46 O6	502,3294	501,3222	501,3213
8,37	unk alkanna	C16 H16 O8 S	368,0571	367,0498	303,0862
6,23	unk alkanna	C17 H10 O6	310,048	309,0407	211,0381
5,95	unk alkanna	C16 H18 O7	322,1052	321,0979	234,0162
5,47	unk alkanna	C17 H14 O3	266,0934	531,1796	363,1344
4,08	unk alkanna	C21 H16 O10	428,0735	427,0662	229,0137
14,14	unk alkanna	C16 H12 O6	300,063	299,0558	225,0543
8,02	norathyriol	C13 H8 O6	260,0319	259,0246	203,0343
17,01	unk alkanna	C26 H52 O6	460,3752	459,3679	459,3686
3,19	unk alkanna	C13 H10 N2 O10	354,0354	353,0282	309,0376
16,96	unk alkanna	C17 H20 O12	415,9829	414,9756	350,9908
0,50	unk alkanna	C14 H16 N2 O6 S2	372,0467	371,0394	159,0424
17,19	unk alkanna	C18 H32 O4	312,2298	311,2226	249,2216
1,10	unk alkanna	C17 H12 O7	328,0584	327,0511	159,0444
16,68	unk alkanna	C21 H24 O9 S	452,1131	451,1059	285,0756
4,86	unk alkanna	C15 H8 O7	300,0271	299,0198	211,0389
16,96	unk alkanna	C14 H15 N3 O4	289,1071	288,0997	211,0392
16,37	unk alkanna	C20 H26 O7	378,1671	377,1598	219,1375
1,27	unk alkanna	C18 H12 O8	356,0528	355,0455	267,0653
8,40	unk alkanna	C16 H18 O6	306,1103	305,103	190,0269
0,72	monohidroxy benzoic acid	C7 H6 O3	138,0356	137,0283	108,021
4,98	unk alkanna	C36 H30 O16	718,1522	717,1449	475,1021
3,42	unk alkanna	C25 H30 N3	372,2384	371,2311	83,0506
6,14	unk alkanna	C10 H6 N4 O S	230,0274	229,0201	157,0289
7,43	unk alkanna	C16 H18 O8	338,0426	337,0353	265,0497
12,81	unk alkanna	C16 H16 O6	304,0949	303,0876	234,0166
5,27	unk alkanna	C10 H6 O5	206,0215	205,0142	177,0189
16,95	unk alkanna	C33 H50 N4 O20 S3	918,2175	917,2101	287,0921
4,26	unk alkanna	C16 H8 O7	312,0631	311,0558	267,0653
17,66	unk alkanna	C22 H46 O9 S	486,286	485,2787	485,2771
16,01	alkannin	C16 H16 O5	288,1	287,0927	190,0266
18,42	unk alkanna	C13 H26 N2 O8 S	370,1408	369,1335	270,0885
18,43	unk alkanna	C47 H72 O27 S3	1164,3427	1163,336	369,1336
15,72	unk alkanna	C15 H14 O4	258,0892	303,0874	247,0243
18,53	unk alkanna	C39 H46 N2 O14	766,2951	765,2878	269,0818
19,61	unk alkanna	C36 H34 O10	626,2146	625,2073	537,1538
18,94	unk alkanna	C32 H30 O9	558,1883	557,181	470,0986
17,40	unk alkanna	C16 H14 O5	286,0842	285,0769	227,0346
19,26	unk alkanna	C34 H30 O10	598,1841	597,1768	537,1545
18,84	unk alkanna	C32 H28 O9	556,173	555,1657	486,0945
19,66	unk alkanna	C37 H34 O10	638,2148	637,2076	537,1546
17,82	unk alkanna	C21 H22 O7	386,1364	385,1291	285,0763
19,49	unk alkanna	C32 H28 O8	540,1777	539,1704	470,0998
19,76	unk alkanna	C37 H36 O10	640,2301	639,2228	537,1546

9.3 INDIAN YELLOW (*MANGIFERA INDICA* L.)

Tab. 9.2 – Main compounds detected in indian yellow

RT	NAME	FORMULA	MASS	M/Z	BASE PEAK
0,704	unk indian yellow	C12 H14 O7	270,074	269,0667	93,0345
0,773	unk indian yellow	C18 H22 O13	446,1053	445,098	113,0243
0,877	unk indian yellow	C12 H6 O11	325,9915	324,9842	169,007
1,014	unk indian yellow	C19 H32 O9	404,2043	403,197	75,0094
1,446	unk indian yellow	C14 H24 N6 O2 S2	372,1413	371,134	71,0138
1,544	unk indian yellow	C13 H16 O7	284,0892	283,0819	107,0504
1,681	unk indian yellow	C16 H16 N10 O7	460,1206	459,1133	85,0296
2,206	unk indian yellow	C14 H20 N8 O9 S	476,1075	475,1003	299,0652
2,628	unk indian yellow	C16 H22 O8	342,1312	341,1239	75,0082
2,649	unk indian yellow	C7 H6 O3	138,0317	137,0244	93,0341
2,897	unk indian yellow	C25 H46 N4 O7	514,3353	513,3281	449,3125
2,912	unk indian yellow	C23 H49 N O12	531,3254	530,3181	61,9892
3,249	unk indian yellow	C14 H22 N2 O4	282,1574	281,1501	83,0507
3,277	unk indian yellow	C16 H12 N6 O10	448,0632	447,0559	271,0232
3,325	unk indian yellow	C26 H42 N8 O4	530,3324	529,3252	449,3114
3,7	unk indian yellow	C18 H26 N10 O3	430,2199	429,2125	75,0096
3,77	unk indian yellow	C25 H42 O14	566,2541	565,2467	389,2188
4,082	unk indian yellow	C16 H24 N10 O3	404,2026	403,1954	85,0299
5,246	unk indian yellow	C25 H39 N7 O3	485,3108	484,3036	83,051
5,296	unk indian yellow	C20 H16 O11	432,0691	431,0618	255,0297
5,355	unk indian yellow	C16 H24 N10 O2	388,2088	387,2015	75,0101
5,865	unk indian yellow	C12 H14 O5	238,0843	237,077	119,0362
5,952	unk indian yellow	C10 H18 O4	202,1202	201,1129	584,1821
6,345	unk indian yellow	C23 H30 N14 O4	566,2569	565,2496	389,2183
6,432	unk indian yellow	C25 H35 N O4	413,2562	412,249	253,8073
6,517	unk indian yellow	C27 H52 N6 O10	620,3747	619,3673	619,3663
6,582	unk indian yellow	C21 H30 O8	410,1944	409,1872	75,0099
6,751	euxanthone glucuronide_euxanthine	C19 H16 O10	404,0814	403,0742	227,0357
6,98	unk indian yellow	C19 H32 O8	388,2099	387,2026	75,0084
6,984	unk indian yellow	C19 H34 O8	390,2249	389,2176	75,0088
7,527	unk indian yellow	C20 H16 O11	432,069	431,0617	227,0339
7,57	unk indian yellow	C13 H8 O5	244,0372	243,03	243,0304
7,724	unk indian yellow	C13 H8 O7 S	308	306,9928	227,0348
8,773	unk indian yellow	C20 H41 N11 O6	531,3244	530,317	61,9884
8,803	unk indian yellow	C9 H16 O	140,1198	139,1125	118,9395
8,938	unk indian yellow	C14 H10 O5	258,0517	257,0444	214,0245
9,468	unk indian yellow	C13 H8 O5	244,0367	243,0294	243,0293
10,186	unk indian yellow	C20 H18 O10	418,0897	417,0824	227,0347
10,23	unk indian yellow	C21 H20 O12	464,0951	463,0878	227,0345
10,523	unk indian yellow	C36 H66 N6 O10	742,4844	741,4771	61,9883
11,099	unk indian yellow	C12 H22 O4	230,152	229,1447	102,9744
11,501	unk indian yellow	C20 H6 N2 O16	529,9702	528,9629	352,9315
12,28	unk indian yellow	C12 H8 O3	200,047	199,0397	143,049
12,471	euxanthone	C13 H8 O4	228,0424	227,0351	171,0446
12,708	unk indian yellow	C20 H31 N13	453,2829	452,2755	61,9874

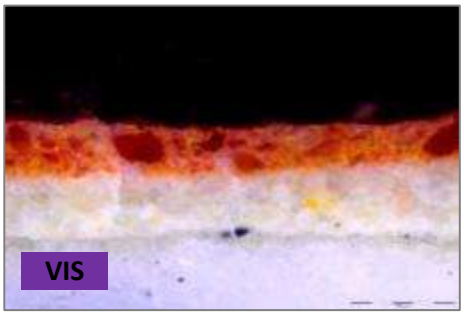
13,108	unk indian yellow	C7 H10 N6 O3 S	258,0533	257,046	214,0279
13,16	unk indian yellow	C28 H34 N4 S	458,2511	457,2439	221,1534
13,234	unk indian yellow	C21 H36 O9	432,2353	431,228	125,0968
13,26	unk indian yellow	C30 H65 N17 O2 S	727,5227	726,5154	680,5199
13,965	unk indian yellow	C25 H15 N11 O4	533,1309	532,1237	356,0945
15,438	unk indian yellow	C15 H29 N O3	271,2143	270,207	57,038
15,496	unk indian yellow	C16 H24 Cl2	286,1247	285,1166	269,0861
15,648	unk indian yellow	C11 H13 N O5	239,0794	238,0721	119,0368
16,004	unk indian yellow	C17 H26 O4	294,1828	293,1756	136,0888
16,378	unk indian yellow	C19 H2 N2 S3	353,9381	352,9308	126,9052
16,543	unk indian yellow	C27 H42 N10 O6	602,3289	601,3216	187,0971
16,604	unk indian yellow	C17 H19 N O4	301,1317	300,1241	149,0961
16,899	unk indian yellow	C13 H26 N6 O5 S	378,1675	377,1602	96,9599
17,114	unk indian yellow	C22 H46 O9 S	486,2856	485,2784	485,2776
17,282	unk indian yellow	C15 H32 O5 S	324,1969	323,1896	96,9603
17,358	unk indian yellow	C17 H36 O6 S	368,2232	367,2159	96,9602
17,386	unk indian yellow	C19 H40 O7 S	412,2493	411,242	96,9603
17,399	unk indian yellow	C21 H44 O8 S	456,2754	455,2681	455,2661
17,443	unk indian yellow	C23 H48 O9 S	500,3017	499,2945	499,2953
17,903	unk indian yellow	C21 H26 N2 O6	402,1777	401,1705	194,0818
17,929	unk indian yellow	C15 H23 N O3	265,1674	264,1602	164,0358
18,059	unk indian yellow	C18 H24 N8 O4	416,1929	415,1856	194,0818
18,29	unk indian yellow	C16 H34 O4 S	322,218	321,2108	96,9603
18,385	unk indian yellow	C21 H27 N O4	357,1937	356,1864	194,0819
18,494	unk indian yellow	C21 H26 N2 O6	402,178	401,1707	194,0813
18,641	unk indian yellow	C23 H38 N8 O2 S	490,2847	489,2774	283,1102
18,645	unk indian yellow	C16 H30 O2	254,2246	253,2173	61,9883
18,924	unk indian yellow	C27 H42 N10 O11	682,3032	681,2959	681,2954
19,069	acido palmitico	C16 H32 O2	256,2462	255,2389	116,9325
19,371	unk indian yellow	C25 H26 N6 O2	442,2117	441,2044	441,203
19,918	unk indian yellow	C25 H46 O2 S	410,321	409,3138	409,3122

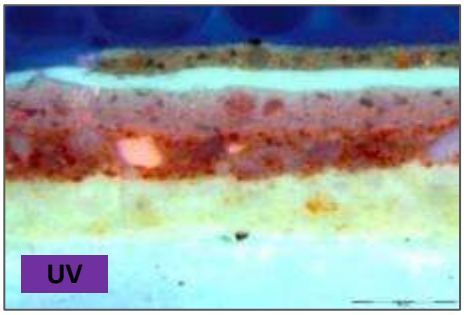
10. CASE STUDIES

10.1 CASE STUDY 1 :

LA MISA DE SAN GREGORIO, PEDRO BERRUGUETE (1450-1504)
Segovia Cathedral, oil on panel, XV century

The sample have been collected from a red area in the vest of the Saint. The sampling dates back to October 2013. In the samples have been identified alizarin, purpurin and munjistin as markers of Madder lake, and azelaic acid related to the use of a siccative oil



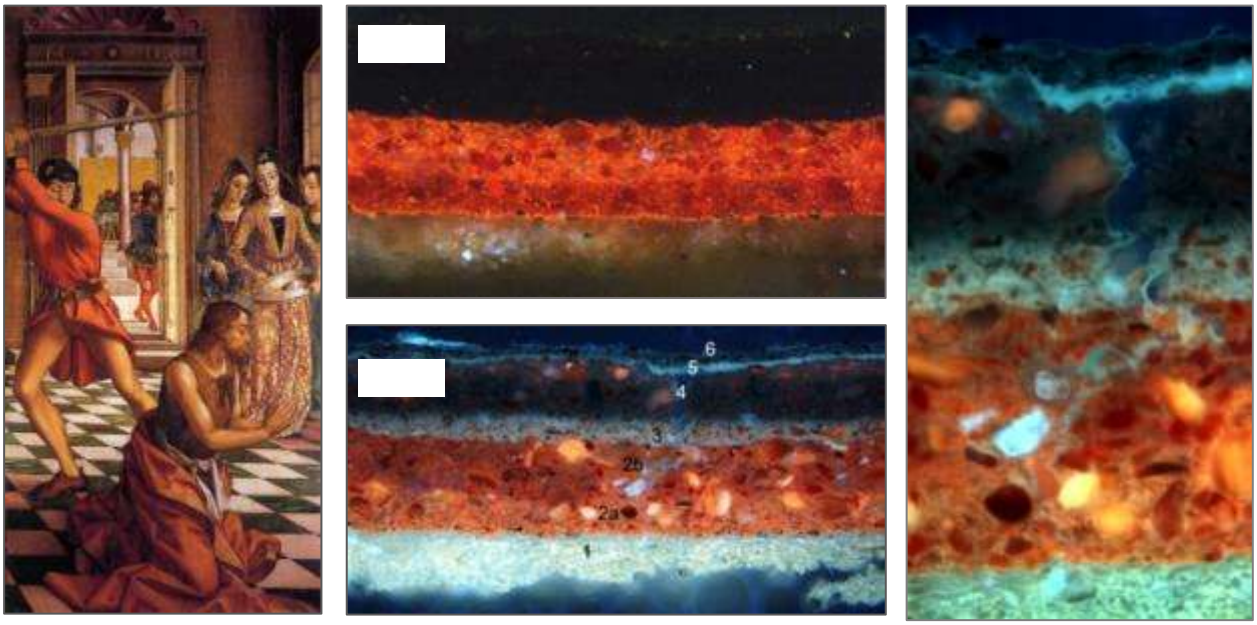


N.	RT	COMPOUND	FORMULA	MW
1	4.37	Azelaic acid	$C_9H_{16}O_4$	188.10486
2	9.36	Munjstin	$C_{15}H_8O_6$	284.03209
3	11.01	Alizarin	$C_{14}H_8O_4$	240,04226
4	13.43	Purpurin	$C_{14}H_8O_5$	256,03717

10.2 CASE STUDY 2

DECAPITACIÓN DE JUAN EL BAUTISTA, PEDRO BERRUGUETE (1450-1504)
Santa Maria del Campo, oil on panel, XV century

The sample have been collected from a red area in the vest of the soldier. The sampling dates back to October 2013. The UV photo showed the typical orange fluorescence of madder and actually in the samples have been identified main and minors compounds considered markers for madder lake from Rubia tinctoria; alizarin, purpurin, pseudopurpurin, xanthopurpurin, munjistin and quinizanin.

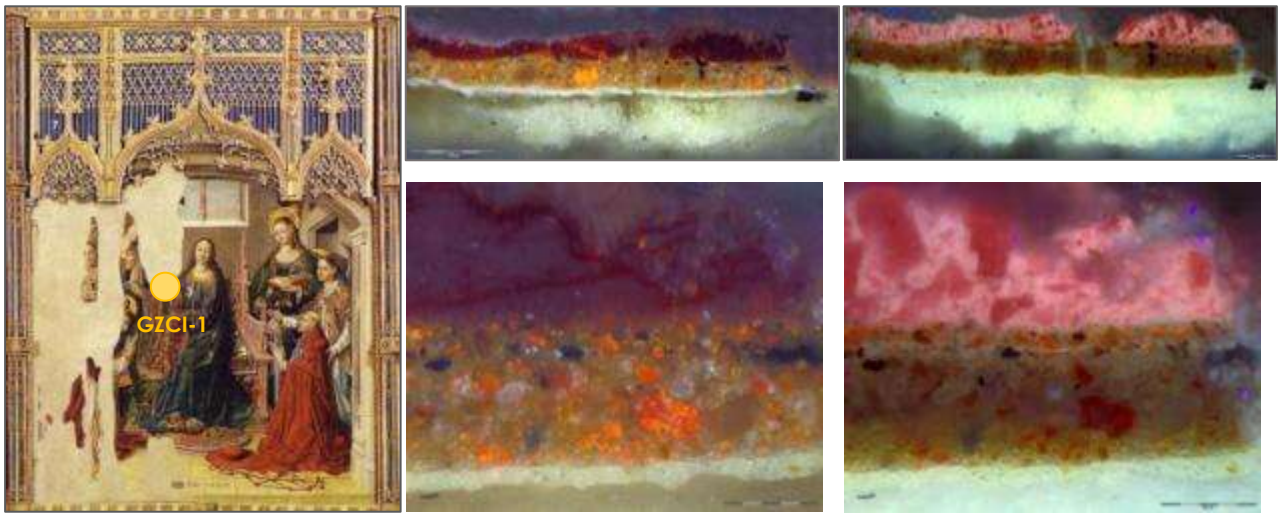


N.	RT	COMPOUND	FORMULA	MW
1	9.25	Anthragallol	$C_{14}H_8O_5$	256,037171
2	9.26	Pseudopurpurin	$C_{15}H_8O_7$	300,02700
3	9.45	Munjistin	$C_{15}H_8O_6$	284.03209
4	9.46	Quinizanine	$C_{14}H_8O_4$	240,04226
5	11.15	Alizarin	$C_{14}H_8O_4$	240,04226
6	13.57	Purpurin	$C_{14}H_8O_5$	256,03717
7	13.43	Xanthopurpurin	$C_{14}H_8O_4$	240,04226

10.3 CASE STUDY 3 AND 4

BAUTISMO DE CRISTO, PEDRO BERRUGUETE (1450-1504)
Santa Maria del Campo, oil on panel, XV century

RETABLO DE S. ILDEFONSO, FERNANDO GALLEGO (1440-1507)
Cathedral of Zamora, S. Ildefonso chapel, oil on panel, XV century



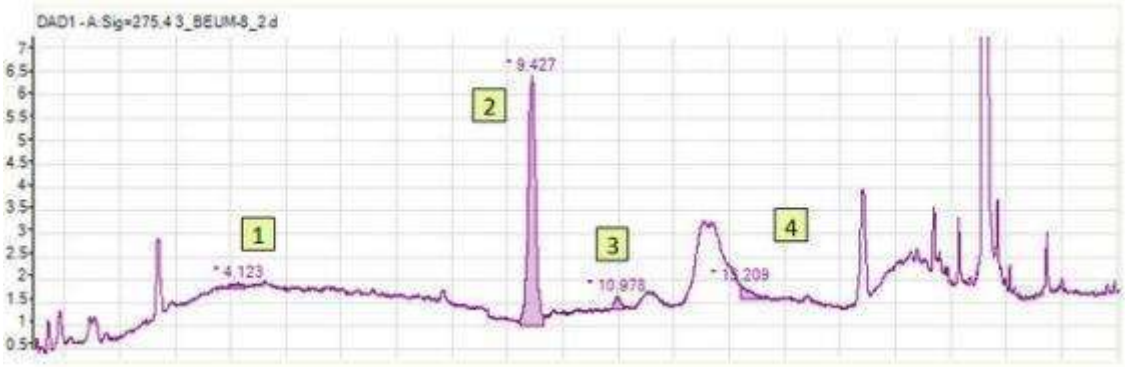
Due to the extremely low amount of samples, in both casees have been possible to identify just alizarin as marker for madder lake.

N.	RT	COMPOUND	FORMULA	MW
1	11.20	Alizarin	$C_{14}H_8O_4$	240,04226

10.4 CASE STUDY 5

NATIVIDAD, FERNANDO GALLEGO (1440-1507)
Diocesan Museum of Salamanca, oil on panel, XV century

The sample have been collected from a red area in the vest of San Joseph. The sampling dates back to October 2013. In the sample have been identified Kermesic acid and an unknown compound (unk 222) attributed to kermes lake. Morover have been detected azelaic, suberic and sebacic acid, which are degradation products of siccative oils.



N.	RT	COMPOUND	FORMULA	MW
1	2.17	Suberic acid	$C_8H_{14}O_4$	256,03717
2	3	unk kermes 222		222,00894
3	4.41	Azelaic Acid	$C_9H_{16}O_4$	188.10486
4	6.77	Sebacic acid	$C_{10}H_{18}O_4$	202,12051
5	7.87	Kermesic acid	$C_{16}H_{10}O_8$	

10.6 CASE STUDY 6

RETABLO DE TRUJULLO

En la muestra ha sido identificado acido carminico como compuesto mayoritario. La contemporánea presencia de acido kermesico, acido flavokermesico, dcIIC, dcVII confirman la identificacion como laca de cochinilla. Al tempo de retención 4,49 se encuentra un compuesto de coloracion rojo/porpora (bandas de absorción en 278, 338, 514 y 558) no identificado (no presente en la base de datos) con m/z 451,3296 a lo cual ha sido atribuida fórmula $C_{22}H_{44}N_4O_4$.

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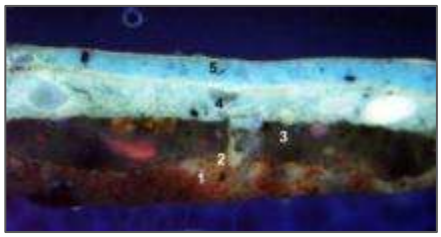
N.	RT	COMPOUND	FORMULA	MW
1	2.46	DcII C-glucoside of flavokermesic acid	$C_{22}H_{20}O_{12}$	476,09548
2	2.72	Carminic acid	$C_{22}H_{20}O_{13}$	492,09039
3	4.45	Azelaic Acid	$C_9H_{16}O_4$	188.10486
4	4.49	unk 452	$C_{22}H_{44}N_4O_4$	452,3348
5	4.96	dcVII carminic acid isomer	$C_{22}H_{20}O_{13}$	492,09039
6	7.91	Kermesic acid	$C_{16}H_{10}O_8$	330,03757
7	7.96	Flavokermesic acid	$C_{16}H_{10}O_7$	314,04265

10.7 CASE STUDY 7

CATEDRAL DE GRANADA

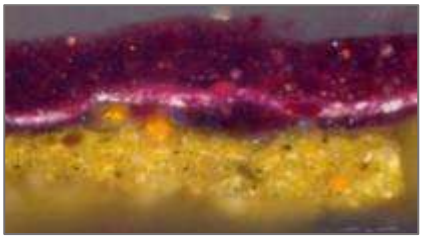


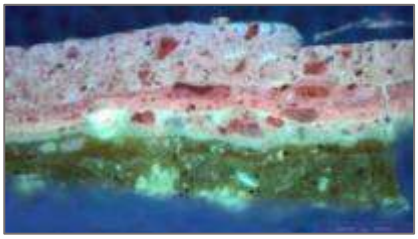
En la muestras ha sido identificado el principale marcador del colorante derivado por el palo de Brasil (Caesalpinia echinata); el Type C. Brasilina y brasileína, los otros compuestos mayoritarios en el Brazil, que todavia suelen ser presentes sobre todo en muestras no envejecidas, no han sidos encontrados. Tambien se han encontrados tres compuestos que señalan la presencia de indigo; hidroxy-isoindigo, isatin y indigotin.

N.	RT	COMPOUND	FORMULA	MW
1	4.13	Type C	$C_{13}H_8O_5$	244,03717
2	6.60	Unk 278 hidroxy-isoindigo	$C_{16}H_{10}N_2O_3$	278,06914
3	6.80	Sebacic acid	$C_{10}H_{18}O_4$	202,12051
4	11	Isatin	$C_8H_5NO_2$	147,03203
5	14.15	Indigotin	$C_{16}H_{10}N_2O_2$	

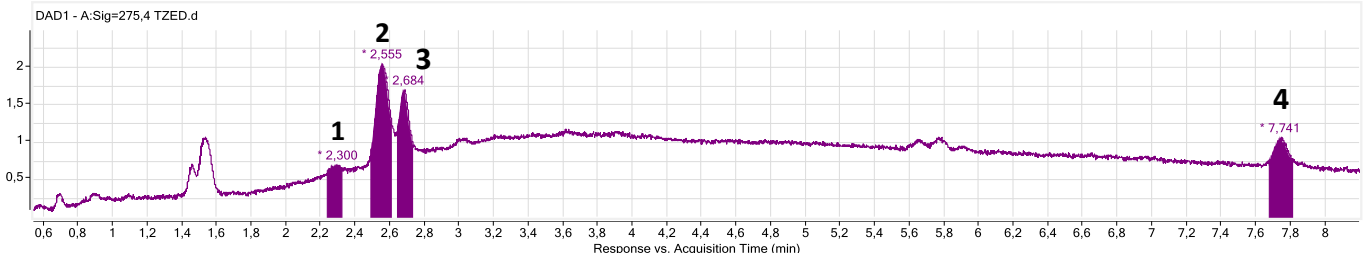
10.8 CASE STUDY 8

EMPERADOR DOMIZIANO, FRANCISCO DE ZURBARÁN, XVI CENTURY





Por comparación con el blanco analitico han sido identificados tres compuestos atribuidos a la cochinilla: dcII, ácido carmínico y ácido flavokermésico. No se encontraron dcIV, dcVII y ácido kermésico, que tambien suelen ser marcadores por esta especie tintóreas. A lo tiempo de retencion 2,7 sale un pico con m/z 582.5835 que no ha sido identificado como un compuesto de la cochinilla ni de otras especies incluidas en la base de datos

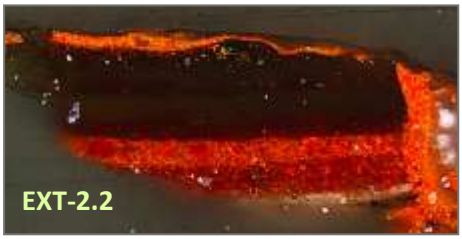


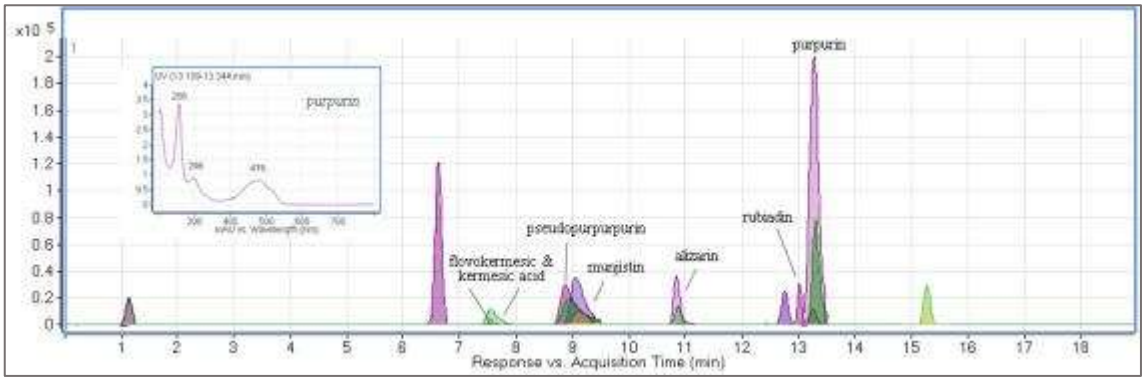
N.	RT	COMPOUND	FORMULA	MW
1	2,3	dcII C-glucoside of flavokermesic acid	$C_{22}H_{20}O_{12}$	476.0956
2	2,6	Carminic acid	$C_{22}H_{20}O_{13}$	492.0904
3	2,7	unk 581		582.5835
4	7,8	Flavokermesic acid	$C_{16}H_{10}O_7$	314.04265

CASE STUDY 9

THE ENTOMBMENT OF CHRIST, MASTER OF PORTILLO
Oil on panel, XV century

The painting has an interesting origin and chronology. It is attributed to an anonymous painter known as "Master of Portillo", working at the end of XV century in the surroundings of Portillo (Valladolid , Spain). The chromatographic analysis demonstrated that the original red lake, detected both in samples EXT2.1 and in sample EXT-2.2, was a madder lake from a Rubia tinctoria. A kermes lake was probably used for the realization of the overpaints of sample EXT-2.2

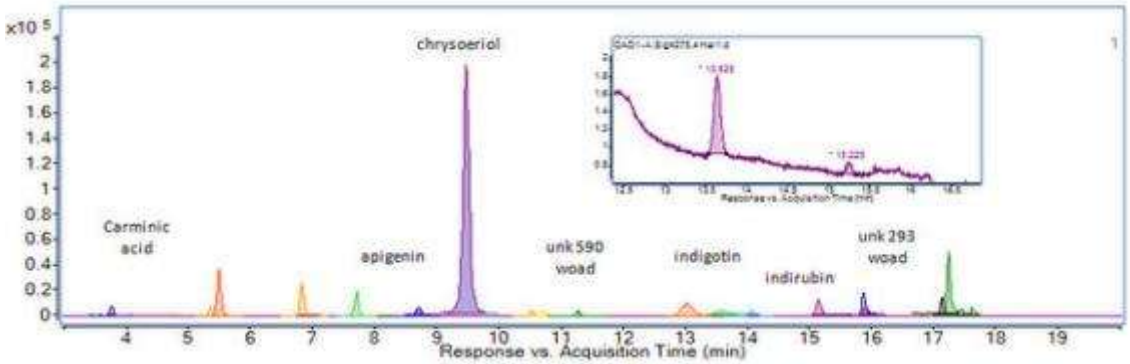




N.	RT	COMPOUND	FORMULA	MW	EXT-2.1	EXT-2.2
1	7.6	kermesic acid	c16h10o8	330,0408		x
2	7.5	flavokermesic acid	c16h10o7	314,0475		x
3	9,25	Anthragallol	C14H8O5	256,037171	x	x
4	9,26	Pseudopurpurin	C15H8O7	300,02700	x	x
5	9,45	Munjistin	C15H8O6	284.03209	x	x
6	9,46	Quinizanine	C14H8O4	240,04226	x	x
7	11,15	Alizarin	C14H8O4	240,04226	x	x
8	13,57	Purpurin	C14H8O5	256,03717	x	x
9	13,43	Xanthopurpurin	C14H8O4	240,04226	x	x

10.4 CASE STUDY 10

SAN FRANCISCO ANTE EL SULTÀN DE EGIPTO
ZACARÍAS GONZÁLEZ VELÁZQUEZ (1763-1834)
Basilica de San Francisco el Grande, Madrid, oil on canvas, XVIII century

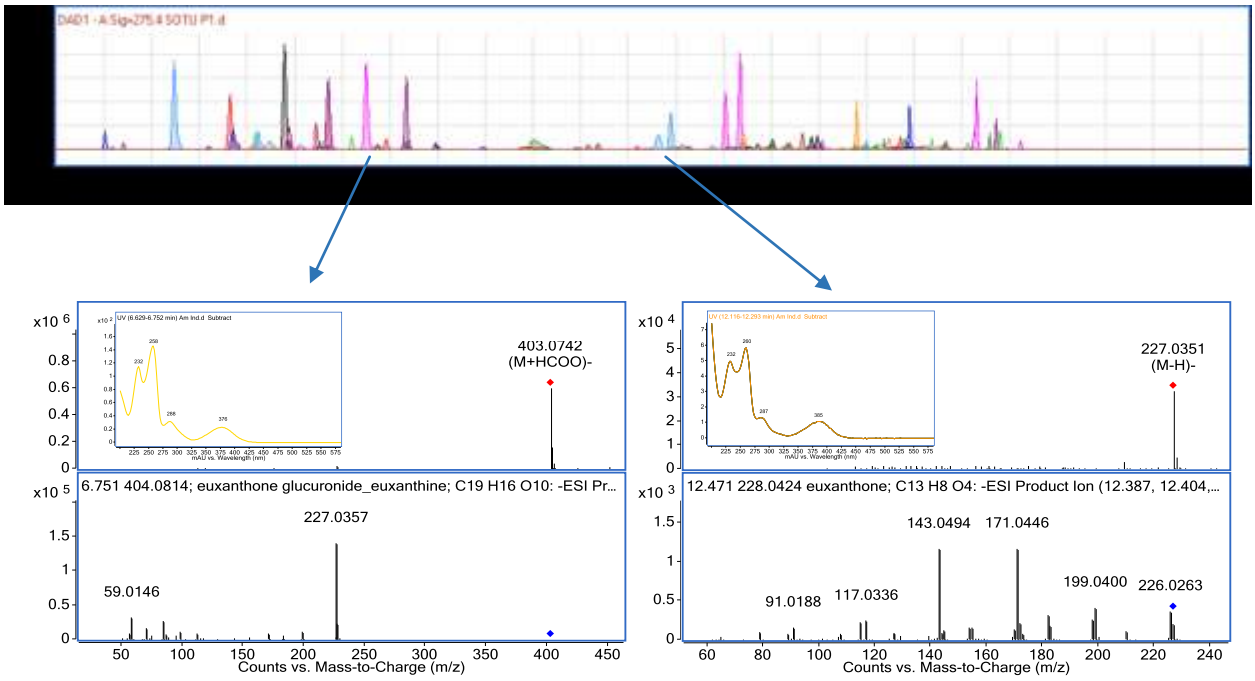


N.	RT	COMPOUND	FORMULA	MW
1	2,72	Carminic acid	$C_{22}H_{20}O_{13}$	492,09039
2	8,42	Apigenin	C15 H10 O5	270,0529
3	9,51	Chrysoeriol	C16 H12 O6	300,0633
4	11,11	Unk 590 woad	C31 H29 N O11	591,1729
5	14,38	Indigotin	C16 H10 N2 O2	262,0745
6	15,72	Indirubin	C16 H10 N2 O2	262,074
7		Unk 293 woad		

CASE STUDY 11

TRANSPORTANDO LA UVA, JOAQUÍN SOROLLA (1863 –1923)
Oil on canvas, 1900

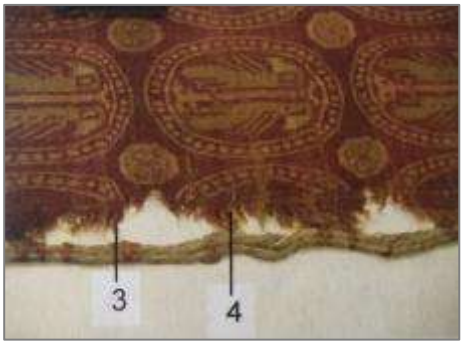
The sample have been collected from a reddish brushstroke in the grapes (lower edge). UV photo showed the typical orange fluorescence of madder and actually a madder lake have been detected. The detection of euxanthine (at 6,7 min) and euxanthone (at 12,38 min) allowed the identification of Indian Yellow, the legendary organic pigment prepared from urine of cows exclusively fed with mango leaves. This is the first documented finding of such pigment in a painting.



CASE STUDY 12

ISLAMIC HISTORCAL TEXTILE (XI CENTURY)
Inglesia de Carrion de los Condes of Palencia

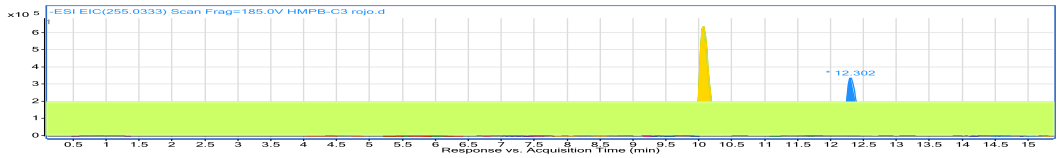
Three fibre samples belonging to an Islamic textile of XI century have been aalyzed in orde to caracterize the colourants used to dye it.

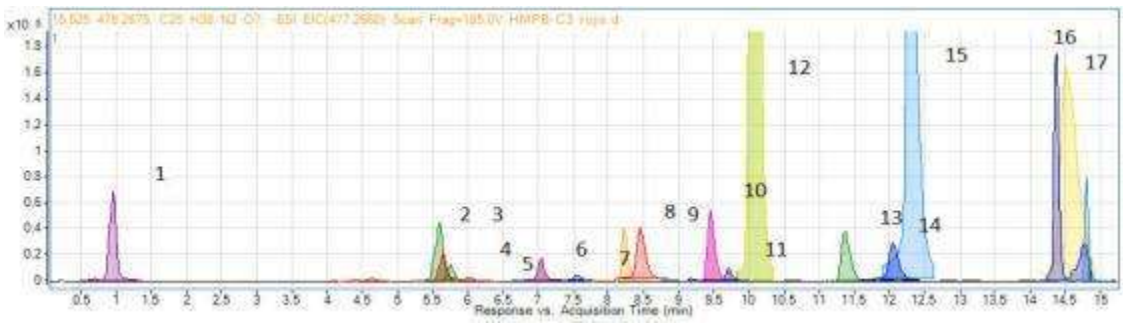


HMPB-C3 HMPB-C4 HMPB-C5

SAMPLE HMPB-C3 : RED FIBRE OF THE FABRIC WEFT

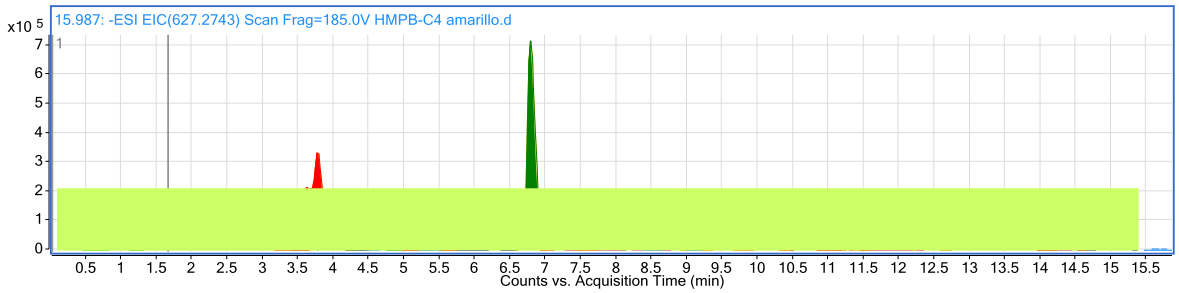
COMPOUNDS				DYE SPECIE
1.	Alizarin primveroside	9.	Anthragallol	**Granza** (Rubia tinctoria L. + Morinda?)
2.	Lucidin primveroside	10.	Rubiadin diglucoside	
3.	Purpurin primveroside	11.	Alizarin	
4.	Purpurin glucoside	12.	Munjistin methylester	
5.	Munjistin?	13.	Xanthopurpurin	
6.	Alizarin di glucoside	14.	Purpurin	
7.	Rubiadin primveroside	15.	Rubiadin	
8.	Morindone	16.	Physcion	

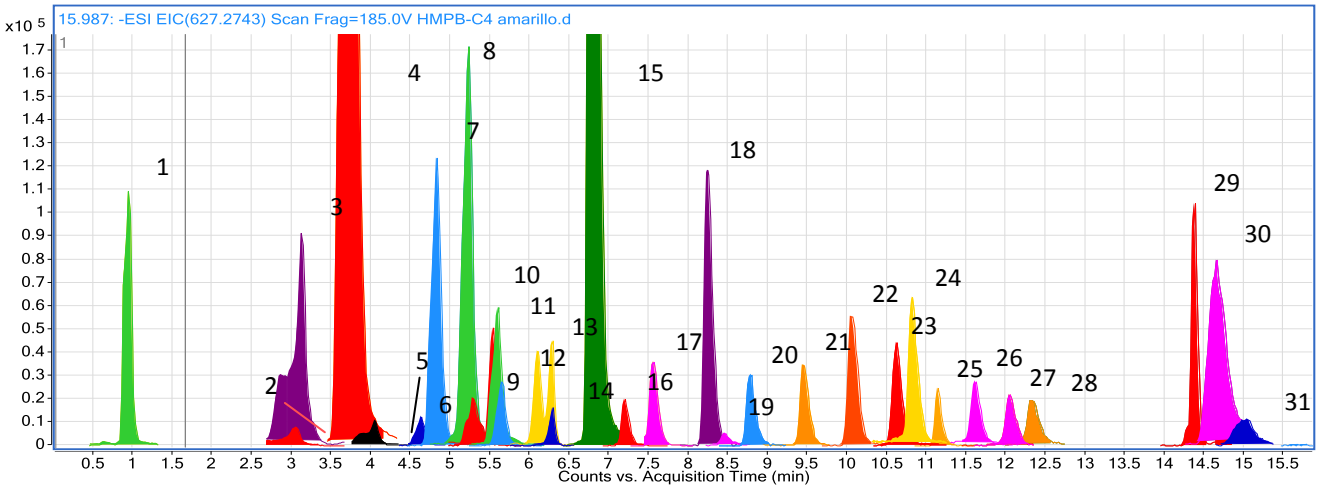




SAMPLE HMPB-C4 : YELLOW FIBRE OF THE FABRIC WEFT

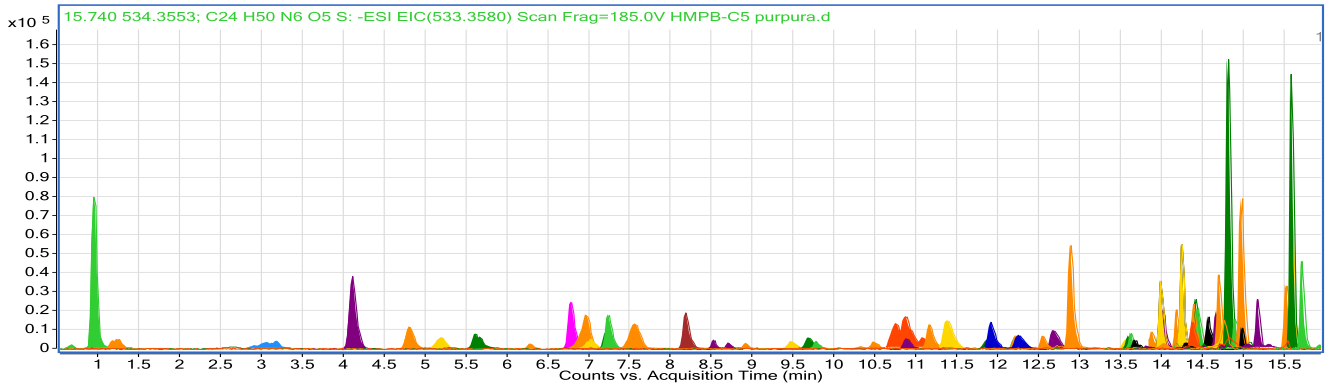
COMPOUNDS		DYE SPECIE
1. Luteolin 7-sulfate-3'-O-glucoside 2. Luteolin 3'-7-di-O-glucoside 3. Luteolin 7-O-glucoside 4. Luteolin di-O-glucoside 5. Kaempferol glucuronide 6. Luteolin-3-O-β-D-glucuronide methyl ester 7. Luteolin-7-O-β-D-glucuronide methyl ester 8. Apigenin-7-O-β-D-glucuronide methyl ester	9. Apigenin 7-O-glucoside 10. Chrysoeriol-7-glucoside 11. Luteolin-O-glucoside 12. Luteolin 13. Unk weld 330 14. Unk weld 262 15. Apigenin 16. Chrysoeriol	**Weld** (Reseda luteola L.)
1. Alizarin primveroside **2.** Lucidin primveroside **3.** Lucidin **4.** Alizarin **5.** Majoronal ?	**6.** Xanthopurpurin **7.** Purpurin **8.** Munistin **9.** RT 27 **10.** Rubiadin	**Madder** (Rubia tinctoria L.)
1. Indigotin 2. Indigorubin		**Indigo**

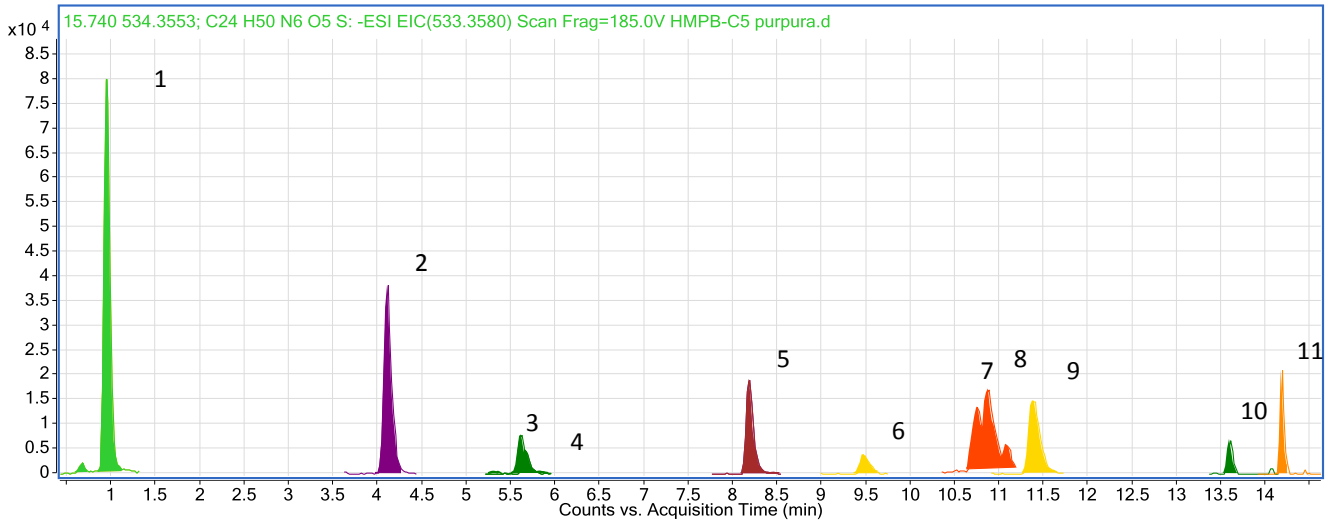




SAMPLE HMPB-C5 : PURPLE FIBRE OF THE FABRIC WEFT

COMPOUNDS		DYE SPECIE
1. Benzoic acid **2.** 3-Hexenyl-β-D-glucopyranoside **3.** β(γ)-hydroxy orcein **4.** unk 329 orchil	**5.** unk 329 orchil **6.** unk 297 orchil **7.** Unk 417 orchil	**Orchil ?** (Roccella tinctoria)
1. Alizarin primveroside (ruberythric acid) **2.** Morindin **3.** morindone		**Madder** (Morinda?)

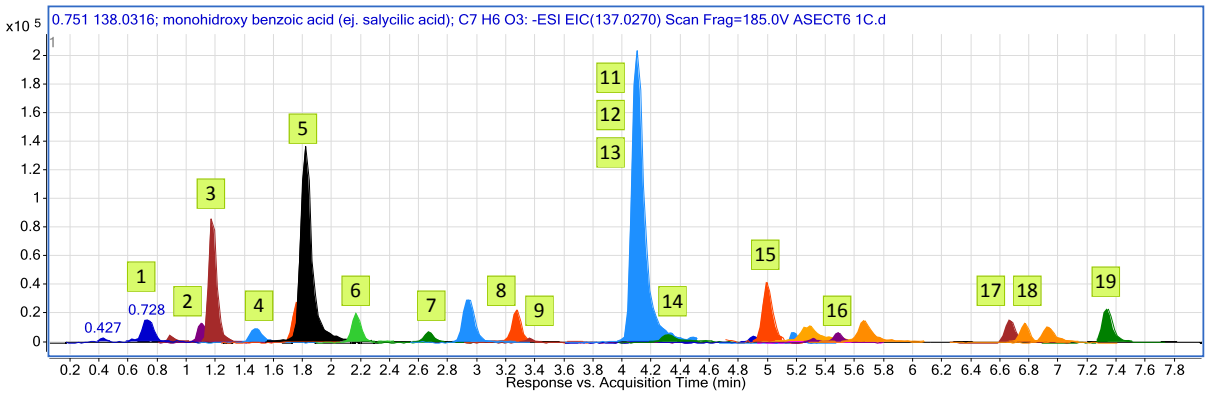




CASE STUDY 13

HISTORICAL TEXTILE (SAMPLE ASECT6-1C)

By comparison with the analytical blank, ellagic acid and a number of its derivatives have been identified as major components, denoting the presence of a tannic dye (Oak?). The presence in the sample of luteolin and its main glucosides (luteolin 3'-7-di-O-glucoside and luteolin 7-O-glucoside) proves use of Reseda luteola (Weld) as yellow dye. Some dicarboxylic acids (azelaic, suberic and sebacic acids) have been detected denoting the presence of an oil. They are in fact common degradation products of siccatives oils. Other two dyes, Carminic acid and indigotine, have been found in low concentration. These compounds, even if present in very low percentages with respect to the other dyes, are clear markers of cochineal and indigo respectively.



N.	RT	COMPOUND	FORMULA	MW
1	0.73	monohidroxy benzoic acid	$C_7H_6O_3$	138,0312
2	1.10	ellagic acid derivate	$C_{15}H_7O_{10}$	345,9944
3	1.17	benzoic acid	$C_7H_6O_2$	121,0348
4	1.48	ellagic acid glucuronide	$C_{20}H_{14}O_{14}$	478,0362
5	1.65	Elagic acid glucuside	$C_{20}H_{16}O_{13}$	464,0569
6	2.17	suberic acid	$C_8H_{14}O_4$	256,03717
7	2.67	carminic acid	$C_{22}H_{20}O_{13}$	434,0465
8	3.27	ellagic acid pentoside	$C_{19}H_{14}O_{12}$	433,0413
9	3.36	luteolin 3'-7-di-O-glucoside	$C_{27}H_{30}O_{16}$	610,2902
10	3.44	Ellagic acid	$C_{14}H_6O_8$	302,0038
11	4.10	unk flavonoid 301	$C_{14}H_7NO_7$	301,0187
12	4.13	luteolin 7-O-glucoside	$C_{21}H_{20}O_{11}$	448,0985
13	4.32	ellagic acid derivative	$C_{15}H_7O_{10}$	342,9931
15	4.41	kampferol glucuronide	$C_{21}H_{18}O_{12}$	462,0783
16	5.48	3-O-Methylellagic acid	$C_{15}H_8O_8$	316,0197
17	6.67	Sulfuretin	$C_{15}H_{10}O_5$	270,0505
18	6.77	Sebacic acid	$C_{10}H_{18}O_4$	202,1183
19	7.33	Luteolin	$C_{15}H_{10}O_6$	286,0473
20	14.11	Indigoti	$C_{16}H_{10}N_2O_2$	262,0745

CONCLUSIONS AND FUTURE DEVELOPMENTS

4.1 CONCLUSIONS AND FUTURE DEVELOPMENTS

The research activity carried out over the last three years resulted in the creation of a complete database available for the routine analysis of natural lakes in Cultural Heritage objects. This data collection includes multi-analytical spectra (MS and MS/MS spectra, UV-VIS Absorption Spectra, Reflectance spectra, FTIR spectra), optical data, colorimetric parameters and multispectral photos (VIS, UV at 254 and 366 nm, FCUV, IR, FCIR). The main objective of the thesis could therefore be considered achieved.

All the compounds isolated in each sample have been recorded and, whenever possible, univocally identified by means of comparison with analytical standards, literature data of study of fragmentation pattern. This data processing phase leaded to the complete fingerprint of all the compounds present in the analyzed samples. The subsequent comparisons among compositional data obtained from lakes belonging to the same tinctorial specie results in the identification of three category of compounds.

The first is composed by those compounds detected in all the samples independently from the applied recipe and for this reason categorized as "markers of the specie". The second category concern those compounds identified in at least two recipes and consequently categorized as "recurrent compounds". These substances have been correlated to the making procedure. Despite this fact confirms the possibility to collect information about making procedures starting from the chemical composition of a lake, the association to a specific treatment/additive or to a specific operative step is not so easy to define. With the purpose to achieve this correlation between chemical composition and a specific aspect of the making procedure, a statistic treatment of data with chemometric tools have been planned in the future months. The solution to the present problem, as it does for most of the issues related to real systems, is in fact tied to the consideration of multiple variables simultaneously. Is therefore required a multivariate approach that allows to take into account all the variables involved, allowing to take advantage of all the information contained in the data to interpret.

In addition to the above mentioned groups (markers and recurrent compounds), other uncategorized compounds have been identified in all the analyzed lakes. These are suppose to have a direct relation with the recipe employed to their production since they identify univocally each sample as a sort of fingerprint. However, often the small amount of available sample prevents the detection of such minor compounds usually directly related to the applied recipe. This doesn't change the fact that the methodology could be successfully applied to those samples that presents higher concentrations of dyes such as, for example, samples from historical pigments collections, Painter's palettes, or Ancient cosmetics.

The most significative result obtained from the study has been the discovery of a set of markers for distinguishing true Indigo (Indigofera Tinctoria) from Woad (Isatis Tinctoria). Even if a number of different samples have been analysed in addition to those originally considered in the study with the aim to confirm the hypothesis, new experiments have been planned in the next months. These new set includes indigo and woad commercial sample from different suppliers worldwide and home-made woad samples prepared starting from plants collected in different locality. The reason of this further investigation is confirm the exclusion of a possible influence of the extraction methods or the geographic source.

High Performance Liquid Chromatography (HPLC) coupled with Diode Array Detector (DAD) and Quadrupole-Time of Flight Mass Spectrometer (Q-TOF-MS) confirmed to be a powerful tool for the analysis of Cultural heritage samples. The present study demonstrated one again the efficiency in detection low concentrations of analytes in pictorial film micro-samples.

ANNEX I

RECIPES FOR THE PRODUCTION OF LAKES

BOLOGNESE MANUSCRIPT

RECIPE N. 70

Ingredients: Verzino, Egg white, Potash alum

Take some scraped verzino and put it into prepared egg white (1,2) for a day and a night (3). It must be completely covered with the egg. Add a little Potash alum (4) and strain the mixture with a piece of linen. Use the coloured liquid to distemper blue pigments.

RECIPE N. 75

Ingredients: Flower of woad, quicklime, water

Take some flower of woad and bake it in a earthenware shovel (1) until it is well burnt. Grind it and mix with 5 parts of quicklime powder and clear water so that to obtain a fluid cream (2-4). Spread it on a smooth table and let it dry in the sun. Break into small pieces and then let it dry again in the sun. Soak the pieces into the first solution and then dry them in the sun or at the fire. Repeat the step if the colour results not enough deep.

RECIPE N. 76

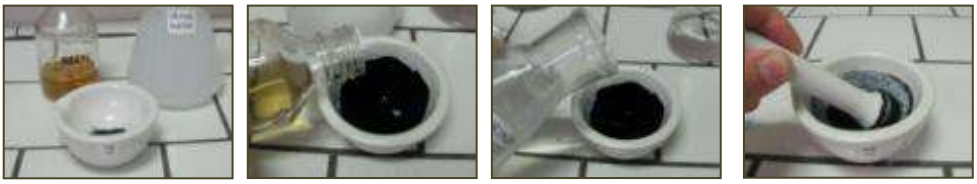
Ingredients: Flower of woad, gypsum, water, potash alum

Take 3 parts of fine gypsum and 6 part of flower of woad. Grind and mix them together so that to obtain a paste. Spread the mixture on a stone or marble table and let it dry. Wet it with alum water and then add other flower of woad. Let it dry and then wet again with the alum water. Finally spread the paste in the stone/marble table and let it dry.

RECIPE N. 77

Ingredients: Flower of woad, Urine, vinegar

Take flower of woad and mix it with urine and strong vinegar so that to obtain a paste. Make a brick and dry it in the sun. If it is too pale add more flower of woad and then dry it again.



RECIPE N. 79

Ingredients: Flower of woad, gypsum, potash alum, water

Take fine gypsum and mix it with flower of woad so that to obtain a coloured watery paste. Wet the woad paste with alum water. Let it remain so until it begins to shrink. Then spread it and let it dry. Wet it up again with the alum water and spread it in a stone or marble table. When nearly dry, cut it into pieces and let it finish drying.

RECIPE N. 89

Ingredients: buckthorn berries, potash alum

Put some ripe berries of buckthorn in a glass vase and press with finger in order to disrupt them. Place the vase under the sun and let it remain until the juice cover the berries (1). Strain the juice pressing well the marc (2) and add 1,3 g of potash alum per every 100g of juice (3). Place the mixture in the sun in a closed vase for three or four days stirring well 3 or four times every day. (4) To use it after long time, distemper with clear lye with a little gum.

RECIPE N. 91

Ingredients: Solanum Nigrum, quicklime, water, Arabic gum

Take some Solanum Nigrum berries and mush them in order to extract the juice (1). Mix the liquor with quicklime (2) and a little gum water (3). Strain the lake and let it dry (4).

RECIPE N. 92

Ingredients: Iris, potash alum, linen cloth

Take some blue Iris flowers and crush them until to obtain a juice (1). Soak some linen cloths in alum water two or three times letting drying in the shade each time (2-3). Bathe the cloths in the flower juice six or seven times and let it dry in the shadow (4). Let it closed in a box and when you want to use the colour soak a piece of dyed linen in water or egg white for a night.

RECIPE N. 93

Ingredients: buckthorn berries, potash alum, linen cloth

Put some ripe buckthorn berries in a glass vase and squeeze well with the hand. Strain the resulting liquid and then use the juice to dye a linen cloth as in recipe 92. Let it closed in a box and when you want to use the colour soak a piece of dyed linen in water or egg white for a night.

RECIPE N. 102

Ingredients: buckthorn berries, white vinegar

Take some ripe and unripe buckthorn berries (1). Boil them with an equal amount by weight of strong white vinegar (2-3). Boil it down to one-half and then strain the mixture with a linen cloth. Preserve the resulting liquor in a glass jar.

RECIPE N. 105

Ingredients: ripe buckthorn berries, lye, potash alum, quicklime

Take ripe buckthorn berries juice and keep it in a close glass vase for 15 days. To use the dyes take an ounce (approx 28 g) of potash alum per every mezzetta (ca 0,6 l) of strong lye. Boil the solution for the space of one "pater noster" and let it cool down (1). Mix together a glass of the alum-lye solution with 1/3 of buckthorn berries juice and let it rest for a night or more (2). Add a little powdered quicklime and mix until obtain a consistent paste (4). Let it dry in the sun. To strengthen the colour bathe the paste into the green lye two or three times.

RECIPE N. 110

Ingredients: Cochineal clippings, ley, potash alum

In a glass vase boil for the space of a "pater noster" 4,50 of cochineal clippings (1) with a strong ley (2). Put the mixture in a strainer covered with a linen cloth and press with finger. Let the ley boil again and then strain it in the containing clippings strainer. Add slowly 1,3 g of powdered potash alum until a thick scumm grow up (3,4). Stirr the mixture until it became cold. Strain with a linen cloth (5) and let the lake dry. Wash it with fresh water in order to remove all the scumm and let it dry again in the shadow (6).

RECIPE N. 111
Ingredients: cochineal clothes, lye, potash alum

Prepare a very caustic lye and put it to boil in a glazed jar. When it boil add a little of quicklime and strain the solution. Put 2,3 g of cochineal clothlets in another glazed vase and add a proper quantity of the above mentioned lye. Mix it well and then put the vase on the fire and let it boil until reduced to one-third. Add 0,8 g of potash alum and make it boil again until is reduced one-third. Strain with a cloth and dry the lake

RECIPE N. 112
Ingredients: Brazil wood, quicklime, water, starch

Take quicklime and let it boil with the appropriate amount of water to cover two finger deep. Mix and let it boil for the space of three "Ave Maria" (1). Let it stand for a night and filter with a linen cloth. Cover with this lime solution some scraped Brazilwood (2) Add a little starch, mix it well (3) and then let it stand for a night. Separate the starch from the water and make a ball with it. Dry the ball in the oven setting the temperature to mid values (50°C) in order not to burn it. Rehydrate the starch ball immerging it in the Brazil wood water for a while and them let it dry in the shadow.

RECIPE N. 113
Brazil wood, lime, egg white

Mix some powdered lime with white of egg and stir it well (1). Let it settle and separate the scum from the rest by filtering. Add some scraped brazilwood to the egg white and let it stand for two days (2).

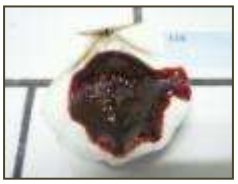
RECIPE N. 114

Ingredients: brazilwood, quicklime, water, potash alum, Arabic gum

Take some quicklime and put it to soak in a vase with sufficient water to cover it three fingers deep (1). Stir until the lime is completely dissolved and let it stand for two days. Separate the resulting water and add to it some Brazil wood (2). Let it settle for three days. Put the whole in the fire and boil it down to one-half (3). Add a little potash alum and Arabic gum. Let it cool and strain with a linen cloth.







RECIPE N. 115

Brazil wood, urine, alum zucharino, lead white, Arabic gum

Soak some scraped brazil wood in cold and purified urine. Add two parts of alum zucharino , one part of lead white and a little Arabic gum and let them rest in the glass vase for two days. Strain the mixture with a linen cloth and put it to dry.





RECIPE N. 116

Ingredients: brazilwood, egg white, potash alum, honey

Put some brazil wood in a glass vase and cover with prepared egg white (1). Add a little potash alum so as "not to make it froth". Then add a drop or two of honey and let it stand for a day. The second day add to the mixture other egg white and potash alum paying attention not to make it froth. Repeat this operation 3 or 4 times. Filter with a linen cloth, put in a shell and let it dry in the sun.





RECIPE N. 118

Ingredients: brazilwood, potash alum, water, Arabic gum, white vinegar

Put some scraped brazil wood in a glass vase and cover with gum water (1). Let it stand in this way one day and one night (2). Boil it until the third part is consumed and then add some potash alum (3). Let it boil for a while and then add 1/3 of strong white vinegar (4). Continue to boil for a short time, filter and dry (5).





RECIPE N. 121

Brazil wood, potash alum, white wine, Arabic gum

Put 13 g of brazil wood in a glass vase and cover it with white wine (2). Let soaking for a day (3) and then add 1/8 of potash alum (4) and the same amount of powdered Arabic gum (5). Let it stand for another day. Boil until the liquid is reduced one-half. Cool down, filter and preserve the in a closed glass bottle.





RECIPE N. 123

Brazil wood, potash alum, red wine, Arabic gum

Take some scraped Brazil wood and put it into a glazed vase with a sufficient quantity of red wine to cover it (1). Close the vase and let soaking for a day and a night in the shadow (2). Expose the solution to the sunlight for 3-4 hours and then add some potash alum and the same amount of powdered Arabic gum (3). Let it stand for 3 or 4 days exposing daily to the sun (never let it out during the night!). Filter and preserve the in a closed glass bottle.





RECIPE N. 127

Ingredients: brazilwood, lye, white wine, potash alum, Arabic gum (1)

Prepare some clear lay, preferably from calcined tartar and white wine. Take some scraped Brasil wood and cover it with the lay. (2) Let it stand for a day and a night and then boil the solution until it reduce the volume to 1/3. (3) Add Arabic gum and let it boil for a while. Add a bit of potash alum and take out from the fire. (4) Cool down and let the lake settle down.




RECIPE N. 130

Ingredients: Shellac, brazilwood, urine, potash alum, white wine, arabic gum, wool

Take 1,5 g of shellac and grind it well using a ceramic mortar (1). Heat some filtered human urine (beforehand let rest for twenty days) continuously removing the froth. Add to the urine some potash alum and boiling the solution going on skimming off (2). Add the powdered shellac and heat the mixture during three "*Miserere*" don't stop stirring (3). Take 1,8 g of scraped Brazil wood and soak it into water (4). Put the vase on the fire and let it boil for a while. Filter the brazil wood solution and let it rest for a natural day. Mix well the two solutions (Shellac and Brazil wood one) and then filter. Prepare a solution mixing 0,6 g of potash alum with 1,15 ml of water and add it to the previous one. Mix it well and let it stand for a day. Filter and dry the lake (5).





RECIPE N. 131

Shellac, urine, potash alum or alum "zucharino", arabic gum

Take human urine and put it to boil continuously removing the froth (1). Let it boil until one half is consumed. Add the powdered shellac and boil it with a small quantity of arabic gum and alum zucharino (or alum) (2). After about 1 hour strain the liquid with a linen cloth. Wait until the lake precipitate and then let it dry by itself (not in the sun or heating) (3). Add to the solution a little powdered calcite (4) and dry the resulting second lake (5).





RECIPE N. 132

Brazil wood, lay, potash alum, lime

Take one-half vase of Brazil wood and put it to soak in strong lay. Let it rest for a night. Put the solution to boil slowly over the fire for a space of a *Ave Maria* (1) and then add the same quantity of other Brazil wood. When the solution is reduced to one-half (2), add a little powdered alum and stir. Take it away from the fire and let it rest and cool down. Filter (3) and let it dry in the sun for a day or two (4, 5). To have a darker pigment add a little lime when the solution is boiling.

RECIPE N. 133

Ingredients: brazilwood, spirit of wine, potash alum, white vinegar, arabic gum

Take 2,7 g of scraped brazil wood and put 1/3 of it in a vase with sufficient spirit of wine to cover it completely (1). Let it soak during a natural day and then add around 0,5 g of powdered potash alum (2). Put the solution in the fire and let it boil for the space of one *paternoster* (3). Strain it and keep both the solution and the scraped barks (4). Take the rest of the Brazil wood and put it to soak in one half vase of white wine adding other 0,5 g of alum and the same quantity of arabic gum. Let it soak for 8 or 10 days and then add the Brazil wood kept from the first infusion. Add a little bit more of alum and let it stand under the direct sun for 4 or 6 days. Strain and keep it in a phial (5). When you want to use the colour mix the two solution in ratio 1:10.

RECIPE N. 135

Reseda luteola, lay, inert

Take 3,2 g of Reseda luteola and add 100 ml of lay. Let it boil until it is reduced four fingers breadth. Put in the dying solution a inert powder and stir well. Strain or let the lake settle.

RECIPE N. 136
Ingredients: brazilwood, rain water, potash alum, white vinegar, white wine, lay

Take 3,2 g of brazilwood and put it to boil in 50 ml of rain water until it is reduced to one half. Add the same quantity of potash alum and let it boil for the space of a *Paternoster*. Keep the first red lake. To make other two lakes let the solution cool down and then put on it some white vinegar or some lay to obtain a red such as cardinals wear.




RECIPE N. 137
Cochineal, Urine, lay, potash alum, rock salt

Put to boil 3 g of cochineal (1) in a sufficient volume of urine or lye for about half an hour (2). Take 1,5 g of potash alum and 1,5 g of rock salt and grind them well with a little lye. Add this second solution to the cochineal bath when it is still boiling. Them put away from the fire and let it cool down (3). Add a little urine and let it rest for 15 days stirring every day. When this period has passed by, filter the solution and dry the lake (4,5).





RECIPE N. 139
Ingredients: Cochineal clothlets, lye, potash alum

Take some cloths dyed with cochineal (1) and put them in a strong lye made from broad beans plants. Let the cloths soaked until complete discoloration (2). Strain and let the lake settle (3). To obtain a thicker pigment admix with a little potash alum (4) and let dry (5).





RECIPE N. 140
Ingredients: Shellac, lye, potash alum

Grind 3,2 g of shellac (1) and put in a very strong boiling lye (2). Let in the fire until dissolution. Then take aprox. 20 ml of water containing 0,6 g of potash alum dissolved. Put the water in a large vase and add the shellac solution (3). Let it stand for two days (4-5) and then filter and dry the lake.

RECIPE N. 184
Ingredients: Saffron, lead white, arabic gum, water

Take a little saffron and a little lead white and distemper them togheter with gum water. Let it stand for half an hour.

RECIPE N. 194
Weld, calcite or lead white, potash alum

Take 3,2 g of weld and cut it in very small pieces (1). Put it in a glass Becker with enough water to cover it (2). Let it boil until reduce to half volume (3). Then take 0,55 g of powdered calcite (or white lead) and 0,13 g of potash alum and admixed them with the weld solution. Let it boil for a while and then take off from the fire and let cool down. The lake will settle in the bottom.

RECIPE N. 203
Brazil wood, potash alum, travertine, lay

Take the same weight of powdered travertine, potash alum and Brazil wood. Put the scraped wood in a strong lay and boil the solution. Add the other ingredients and going on boiling until one-half on the solution is consumed. Strain and dry the resulting lake.

RECIPE N. 15

Iris flowers, burnt potash alum, lemon juice, soda ash

Take fresh purple Iris (1) and pound them in a mortar with lemon juice (2). Add a little potash alum and soda ash. Mix well untill a brillant green colour appear. Filter and let dry in the shadow.

RECIPE N. 19

Gamboge dye, lemon juice, burnt potash alum

Take some gamboge dye (2) and admix it with lemon juice (3) and potash alum (4). Mix until obtain a homogeneous cream and let it dry in the shadow (5).

RECIPE N. 29

Ingredients: unripe buckthorn berries, lye, potash alum

Take some buckthorn berries and bruise them coarsely in a mortar (1). Put the powder in a vase with enough lye to cover it. In order to keep the solution clean, as an alternative put the powder in a paper bag and soak it in the lye (2). Put it to boil in a slow fire until half the lye is consumed (3) and then strain through a linen cloth (4). Add a little potash alum and heat the solution without reaching the boiling point. Put into a shell to dry (5).

RECIPE N. 31

Ingredients: ripe buckthorn berries, lye, potash alum

Take some buckthorn berries and put them in a vase with water in which potash alum has been dissolved in the proportion of 1 part of alum to 6 of berries (1). Let them soften during 7-8 days (2). Put the vase in the fire and let it boil until nearly half the water is consumed (3). Strain (5), put the liquor into bladders and dry in the sun or smoke.

RECIPE N. 65

Ingredients: iris flowers, potash alum, linen cloth

Take some purple iris and separated the petals from the other part of the flower (stamens and stigma). Mash the petals in a mortar until they are well bruised (1) and let them to ferment. (2) Filter the fermented sludge by putting it into a linen cloth and squeezing until all the liquor come out (3). Add potash alum as you please (4) and incorporate well to the iris liquor (5). Leave them rest 5 or 6 hours and them filter again through a linen rag into a shell or vase (6). Let it dry in the shadow.

RECIPE N. 77

Ingredients: brazilwood, distilled water, potash alum

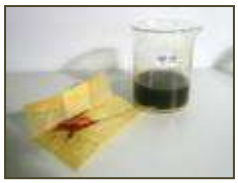
Take brazilwood chips and soften them by soaking into distilled water during three days (1). Boil it until the water is reduced to an half and them separate the coloured liquor from the wood chips (2). For every pound add 1 once of potash alum and a little amount of Arabic gum. Mix it well (3)and then boil it until all the ingredients are dissolved (4). Let it decant and dry the lake (5).

RECIPE N. 79
Ingredients: ripe buckthorn berries, buckthorn yellow, saffron

Take some buckthorn berries and pound them (1). Boil them in a vase of alum water (2-3). To obtain a beautiful green colour, filter and admix to the liquor some buckthorn yellow (4) and a little saffron (5).







RECIPE N. 86
Ingredients: purple iris, quicklime, potash alum

Take purple iris and separate the most highly coloured petals. (1) Leave the flowers to ferment for a day and then mash them in a ceramic mortar (2). Put the juice in a cup. then tie up some quicklime and potash alum in a linen cloth and put it into the juice (3-4). Stir until the green colour is developed and then dry the lake (5).





RECIPE N. 87
Ingredients: brazilwood/campeche, egg white, burnt potash alum

Take scrapples of brazilwood and soak it in a sufficient quantity of egg white to cover it (1-2). Take 2/8 of burnt potash alum and put in a glass vase together with the coloured egg white (3). Stir it well until it result well coloured and them strain through a linen rag. Expose to the sun and let it dry. Do the same with Campeche wood to obtain a purple lake.





RECIPE N. 98
Ingredients: brazilwood, lime water, flour,

Take an handful of brazil wood chips and infuse them in lime water (1). Add some flour to the coloured solution, so that to obtain a thick paste (2-3). Dry it in a oven not too hot (4). Grind it and then mix the powder with a little lime water to create a new paste. Dry it in the shade (5).

RECIPE N. 112
Ingredients: purple iris, lime, sugar

Take some purple iris and mash the petals in a mortar (1). Filter the sludge with a linen cloth in order to obtain a clear liquid (2-3). For two glasses full of it, add lime and potash alum in the quantity of a spatula tip (4). Add a little pulverized candied sugar and stir well in order to dissolve and incorporate all the ingredients. Dry the colour by exposure to the air (5).

RECIPE N. 113
Ingredients: lac, crystalls of tartar, water

Take 2,8 g of lac, 0,2 g of crystals of tartar pulverized and 50 ml of hot water. Dissolve the tartar in the water (2). Grind the lac in a mortar, put the powder in a linen rag and tie it into the form of a little bag (3). Soak the bag into the hot water and place the becker on the fire (4) until water becomes well coloured. Remove the bag from the becker and continue heating the solution until evaporate the water and condensate the lake on the bottom. Filter and dry (5).

RECIPE N. 115

Cochineal, potash alum, water, fennel seeds

Take 2,8 g of cochineal, 0,7 g of potash alum and approximately 100 ml of clear water. Boil the water with 0,7g of fennel seeds until it is reduced one-third (1). Add the cochineal finely powdered and boil during 15 min (2). Then add the powdered potash alum and boil for another quarter of hour (3). Take off from the fire, strain through a linen cloth (4) and let it decants during 8 days (5). Remove the water and dry the precipitate on a shell.





RECIPE N. 116

Cochineal, potash alum, lye

Take 12 grains of cochineal and powder it very fine (1). Add the powder to 50 ml of lye and leave it in infusion for about 2 hours (2). Strain and put the solution on a weak fire (3). When it boils add a teaspoon tip of potash alum and mix it well (4). When the solution forms a thick red scum, filter it quickly through a linen cloth so that separate the recent formed coagulum. (5). Dry the lake and cut it into tablets.





RECIPE N. 127

Ingredients: ripe buckthorn berries, potash alum, lye

Take ripe buckthorn berries (1), mash them with a little powdered potash alum and put the resulting sludge in a glass vase. Let macerate in the sun during two days (2). Add clear lye (3) and boil them over a slow fire until reduced two fingers breadth. Strain the liquor and put it on a bladder. Expose the bladder to the air and let it dry (5).





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RECIPE N. 133
Ingredients: unripe buckthorn berries, potash alum, distilled water, gypsum

Take unripe buckthorn berries (1) and boil with distilled water until the solution is loaded and intensely coloured (2). Add a little potash alum (3) mix well until dissolve it and then and strain the liquor (4). Mix with gilder's gypsum to obtain a pasty cream (5) and let it dry in the shade. To obtain a darker colour, boil a little the solution before adding the gypsum.

RECIPE N. 137
Ingredients: ripe buckthorn berries, potash alum, distilled water

Mash an handful of ripe buckthorn berries in a glass vase (1) and put to boil the resulting liquor (2). Add a little potash alum (3) and continue boiling the solution until a beautiful green colour appear (after about an hour). (4) Take off from the fire, let it cold and filter the liquor (5). Dry and conserve it into a bladder.

RECIPE N. 323 (MARCIANA MANUSCRIPT)
Ingredients: brazil wood, lye

Take a quantity you please of scraped brazil wood and put into lye. Leave the wood in infusion for two or three days to permit the complete extraction of the colouring matter (1), and then strain through a linen cloth (2). Dissolve in the solution a little potash alum and a sufficient quantity of Arabic gum to give the colour body (3). Expose to the sun for 3 or 4 days stirring it occasionally. The longer it is exposed to the sun, the thicker it will be (4). Let it harden to conserve it. To use it distemper with a little lye.

RECIPE N. 9

Ingredients: flower of woad, starch, urine, vinegar

Take flower of woad and mix together with urine and vinegar to obtain a thick paste. Made it into pellets and dry in the sun.



RECIPE N. 12

Ingredients: brazilwood, lead white, potash alum, urine

Take some brazil wood and scrape it into very small chips (1). Distemper a little lead white and a little potash alum into a proper quantity of urine (sufficient to cover the brazil wood). Soak the chips (2/3) in the urine and let in this state until the lake is formed (4). Let dry in the shade.




RECIPE N. 14, 15

Ingredients: scarlet cloth, lye, potash alum

Take some cuttings of fine scarlet cloth and soak them in a strong lye (1). Boil the solution until the whole is dissolved (2) and then add some potash alum (3-4). Add some chips of brazil wood and a little Arabic gum. Mix it well and then made the resulting paste into small pellets which must be suffered to dry. Lake 15is prepared in the same way except that no brazil wood is added.





RECIPE N. 20

Ingredients: brazilwood, lead white, potash alum, urine

Take some scraped brazil wood and boil it together with lime water and potash alum.

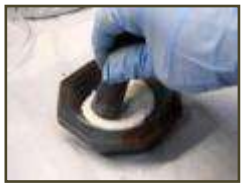
RECIPE N. 101
Ingredients: brazil wood, white lime,

Put a piece of white lime about the size of an egg into water to dissolve. Let it stand for three days and tree nights (1). Add some scraper brazil wood and let in infusion for about one hour (2). Put on the fire and boil until achieve a thick consistency (3). Add a spatula tip of isinglass or turpentine and remove from the fire. Take a little potash alum and mix to the mixture until complete dissolution. Strain and dry the resulting lake.



RECIPE N. 108
Ingredients: brazil wood, calcite, potash alum

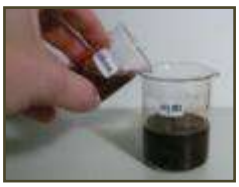
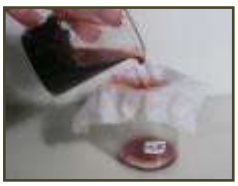
Take brazil wood and scraped it finely. Grind a little potash alum with some powdered calcite (1). Soak all these ingredients in lye and let them stand for a day (2). Mix the whole well and put in the fire (3). Let it boil during a quarter of an hour. Filter through a linen bag/cloth and dry the lake (4).




RECIPE N. 181
Ingredients: brazil wood, red wine, lac, urine, potash alum

Take scraping of brazil wood and let it boil over the fire in a becker full of red wine (1-2). Add a little lac dye distempered with urine (3) and let them boil together. Strain the mixture (5). Add a little potash alum, put again on the fire and stir well to dissolve it. Remove from the fire and pour the content into a basin. Let it dry in the sun.



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