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Case study

# Investigation of natural dyes in 15th c. documents seal threads from the Romanian Academy Library, by LC-DAD-MS (triple quadrupole)

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ABSTRACT

Dyes and biological sources in 40 samples from red seal threads in 38 documents issued by the Chancery of Moldavia between 1460 and 1503 were investigated by liquid chromatography with UV-Vis (DAD) and mass spectrometric (MS) detection. Lac dye (*Kerria lacca* Kerr), redwood type (*Caesalpinia* spp.) and madder (*Rubia* sp.), as individual dyes or in combinations, were responsible for the colour in all the dyed yarns while tannins were present in more than half of the total number of samples. The presence of major dyes, such as alizarin, purpurin, laccaic acid A and soluble redwood — a marker compound for *Caesalpinia* species were observed by both DAD and MS detectors while minor compounds (rubiadin, anthragallol, xanthopurpurin, munjistin, flavokermesic acid etc.) were only detected by mass spectrometry. Single stage MS detection was used in the Full Scan mode followed by data processing through Ion Extraction according to the molecular ions of compounds in the database. Tandem MS detection ($MS^2$) was also achieved, through using the Product Ion Scan operating mode. Identification of dyes was made according to retention time, UV-Vis and MS data, based on information collected on standards — dyes and dyed fibers. The biological sources detected are discussed as compared with those identified in ecclesiastical embroideries from the same period, ordered by the same Prince, Stephan the Great (1457–1504).


## 1. Introduction

Natural dyes were the only source of color from Antiquity and until the synthetic dyes were introduced to the market, in 1856. Initially used only locally, later subject of commercialization, identification of natural dyes in historical textiles may add consistent information about the period and place an object was made. Such information is based on literature records about the natural dyes first trade, new commercial routes or regulation of use [1–3].

The interest for natural dyes in textiles from Romanian collections turned up in the identification of dyes and biological sources in the most representative local ones, dating from the 15th to the 20th century [4–9]. A special interest was given to a collection of textiles from the long reign of Stephan the Great (1457–1504), the ruler of Moldavia for almost 50 years in the second half of the 15th century [10]. Investigation of red dyes in twelve ecclesiastical textiles with donor inscriptions, from this period, preserved at Putna Monastery, showed that the combination of lac dye and madder was used for most of the objects, while kermes, the most expensive red dye at that time [11] was preferred for the more important objects, as those especially designed for Stephan's wife, Princess Maria of Mangop [6,12,13]. A collection of documents with hanging seals emitted by the Chancery of Moldavia in the time of Stephan the Great is preserved at the Romanian Academy Library (BAR). The documents were studied from several perspectives but never as regards the colour source in the hanging seals red silk threads [14].

High performance liquid chromatography with UV-Vis detection (DAD) is the standard set up for investigation of natural dyes, since its development by Wouters, in 1985 [15–21]. In the last 15 years, mass spectrometers were increasingly exploited, in various configurations [22–28]. Their use increases the level of certitude in identifications by introducing new criteria — the molecular ion (for single stage MS detectors) and the product ion scan mode (for tandem MS instruments). MS detection provides lower detection limits for most of the dyes used in historical textiles. Such increased sensitivity is obtained by the reduction of the noise level,

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through exploiting MS instruments in enhanced selectivity modes [28]. Moreover, the MS/MS configurations, such as ion trap and triple quadrupole, were proved as useful instrumentation to reveal the molecular structure of unknown dyes [29].

An analytical protocol for dyes characterization and identification by LC-DAD-MS (ion trap) was recently developed for the first time in Romania [30] and was used for the characterization of a significant number of dyes in textiles from Romanian collection [7,8,31–33].

The present work discusses the results obtained by applying an adapted version of the above-mentioned analytical protocol to the investigation of dyes in red silk threads from 38 "documents with hanging seals", emitted by the Chancery of Moldavia in the time of Stephan the Great.

## 2. Materials and methods

### 2.1. Samples and sample preparation

Forty red silk samples about 0.5 cm long (~3-mg) from 38 documents emitted by the Chancery of Moldavia in the time of Stephan the Great (1457–1504) were available for dye analysis. Three red samples from 2 earlier documents emitted by the same Chancery, in the time of Alexander the Good (1400–1432), were also available (Table 1).

For dyes extraction, samples were transferred in 1.5-mL Eppendorf tubes and 250-μL from a mixture 37% HCl/$CH_3OH$/$H_2O$ 2:1:1 (v/v/v) were added to each fiber. The tubes were placed in a heating block (Thermo Shaker TS-100, manufactured by Biosan) and kept for 10 minutes at 100 °C, according to the method developed by Wouters [15]. The tubes were then moved in a vacuum desiccator (constant pressure at 50-mbar) and the solution was evaporated to dryness at room temperature. Each sample was re-dissolved in 100-μL $CH_3OH$/$H_2O$ 1:1 (v/v) mixture. After 10 minutes of centrifugation at 6000 × g, the supernatants were transferred in chromatographic vials and placed in the automatic injector tray holder.

### 2.2. Database

An "in-house" developed database, relying on retention, UV-Vis and mass spectrometric data, was used for dyes identification. Information about dyes is based on analysis of standards. Identification of the most probable biological sources was made according to data collected on standard dyed fibers and information available in literature [1,2,15]. Retention, UV-Vis and mass spectrometric data of the dyes identified are illustrated in Table 2.

### 2.3. Instrumentation

Samples were analysed using an Agilent 1260 LC system composed of the following modules: quaternary pump (Model G1311C), automatic injector (G1367E) and column thermostat (G1316C). The system was equipped with two detectors serially connected: a diode array detector (G4212A) and a triple quadrupole mass spectrometer (G6410B) equipped with an atmospheric pressure electrospray ion source (ESI, Model G1948B), operated under negative ion monitoring mode.

### 2.4. Chromatographic separation

Separation was achieved on a Zorbax C18 column, 150-mm L × 4.6 i.d., 5-μm particle size, thermostated at 40 °C. The mobile phase consisted of a mixture of aqueous 0.2% (v/v) formic acid (solvent A) and methanol/acetonitrile 1:1, v/v (solvent B). Gradient elution was applied according to the following profile: at 0 min, 15% solvent B; from 0 to 5 min, linear increase to 25% solvent B; from 5 to 10 min, linear increase to 55% solvent B; from 10 to 16, linear increase to 100% solvent B; from min 16 to 18, constant at 100% solvent B; and step jump at 15% solvent B, with a 4 min re-equilibration step. The flow rate was set at 0.8 mL/min. The injected volume was 5-μL, from a total amount of 100-μL resulting from the sample preparation stage. Several injections from the same solution may be performed, as described in the "Results section" below.

**Table 1**
List of documents and their dating. Documents issued in the time of Stephan the Great, dated between 1460 and 1503 are listed first, according to their inventory numbers, while below are presented the two documents from 1409 and 1425, issued in the time of Alexander the Great.

| Inventory number | Date |
|---|---|
| 15 | 1491, January 17 |
| 110 | 1499, December 6 |
| 119 | 1456, September 18 |
| 127 | 1472, June 5 |
| 147 | 1493, March 15 |
| 159 | 1466, September 11 |
| 160 | 1480 |
| 161 | 1497, February 25 |
| 170 | 1498, November 20 |
| 173 | 1493, March 7 |
| 191 | 1499 |
| 194[a] | 1479, March 9 |
| 198 | 1492, October 14 |
| 200 | 1497, March 10 |
| 204 | 1461, August 8 |
| 231[b] | – |
| 236 | 1468, September 24 |
| 243 | 1460, December 5 |
| 245[c] | 1470, May 28 |
| 246 | 1499, August 15 |
| 265 | 1472, January 25 |
| 290[c] | 1470, September 25 |
| 299 | 1494, February 27 |
| 300 | 1497, January 20 |
| 315 | 1490, January 14 |
| 375 | 1490, October 14 |
| 382 | 1502, March 17 |
| 384 | 1491, October 31 |
| 478[d] | 1479, |
| 480 | 1487, October 8 |
| 482 | 1475, April 14 |
| 502 | 1499, November 17 |
| XL/15 | 1503 |
| LXIV/7 | 1488 |
| LXXV/160 | 1479 |
| CI/66_1 | 1495 |
| CLIX/19 | 1502 |
| DCXLIII | 1500 |
| 137[e] | 1409, September 16 |
| 144[e] | 1425 |

[a] Fake document, original seal.
[b] Only seal, no document.
[c] Fake document.
[d] Seal detached from document.
[e] Document issued in the time of Alexander the Good.

### 2.5. Detection

UV-Vis spectra were acquired over the 190–640-nm range, with a resolution of 2 nm. MS detection was made in negative ion monitoring mode with the following ESI operating parameters: drying gas temperature 350 °C; drying gas flow 8 L/min; pressure of the nebulizer gas 40 psi; Vcap 2500 (−). For the single stage MS mode, the triple quadrupole was using the MS2 type Scan; the data storage was set on profile and the peak width at 0.07; fragmentor 135 V; ΔEMV 400 V; The scanning interval was between 100–600 m/z, accelerated voltage on the collision cell: 7 V; Dwell Time 500 ms. In the tandem MS working mode, product ion scan was used, with

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