

# NEW DEVELOPMENTS IN APPLICATION OF HPLC IN ARCHAEOMETRY OF TEXTILES

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## INTRODUCTION

Various aspects of social and cultural history can be revealed through the dyes used for the textiles. The identification of dyes in historical and archaeological textiles is usually based on comparison with known references, quite often however the chemical profile obtained for the sample extract does not match the references available. This can be caused due a number of reasons, including chemical degradation of dyes by light, oxidation or moisture, a limited reference set or due to inefficient extraction process. Maximizing the amount of information remaining in archaeological textiles is therefore needed and requires application of very sensitive analytical methods, including HPLC with different kinds of detectors [1,2].



Cap from a grave dated to the late 17th Century, peat-bank at Gunnister in Shetland, Scotland

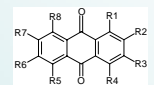


Coat from a 17th Century clothing found on the skeleton of a man buried in moss at Quinfall Hill, Barrock, Caithness, Scotland

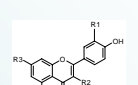


Big, patched textile from the woolen relics found on a skeleton in the Dava Moor, Cromdale, Scotland (16th C/17th C?)

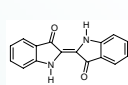
## CONSIDERED DYES - CHEMICAL STRUCTURES



RED DYES - ANTHRAQUINONE DERIVATIVES



YELLOW DYES - FLAVONOIDS



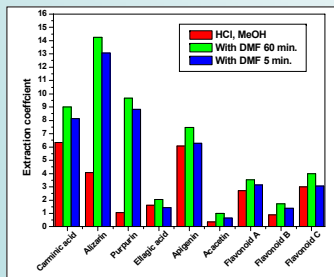
BLUE DYES - INDIGOTIN DERIVATIVES

## CHROMATOGRAPHIC CONDITIONS

The separations were carried out at 30°C on Hypersil BDS C-18 3 µm (100 x 2.1 mm) column with eluent flow rate equal 0.3 ml/min. The gradient of acetonitrile in water with constant amount of 1% water solution of methanesulfonic acid was applied. For methanol-water method the mobile phase consisted of gradient of methanol in water with constant amount of 1% water solution of methanesulfonic acid. The post-column reagent flow rate was 0.1 ml/min, and the reaction volume (tee plus capillary) was 5 µl.

## OPTIMIZED EXTRACTION PROCEDURE

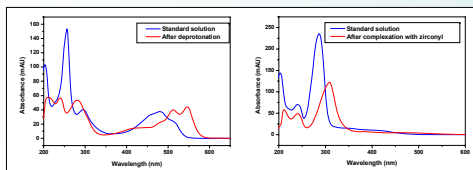
To 100 µl of conc. HCl/MeOH/H<sub>2</sub>O (2:1:1 v/v) solution 1 mg of the sample thread was added, heated on a water bath for 10 min, and then dried in a vacuum desiccator. The examined thread after that was dissolved in 20 µl of DMF/MeOH (1:1 v/v) solution and left for extraction in room temperature for 60 min, filtered using polypropylene syringe filters (0.2 µm) and 5 µl of obtained extract was injected into the chromatographic column.



Comparison of efficiency of following extractions: standard HCl/MeOH/H<sub>2</sub>O, MeOH/DMF extraction performed after hydrolysis in room temperature for 60 min, and for 5 min.; Coefficient = (peak area / mass of the thread taken to extraction [g]); results from the woolen threads dyed contemporary with cochineal, weld, silver birch and sawwort.

## Structure elucidation based on UV/Vis spectra from DAD

Post-column modifications with borax buffer solution at pH 12.4 and 0.1 M zirconyl solution, leading to changes in the UV-Vis spectra of several dyes and their degradation products, can be used to obtain some structural characteristics of the analytes which might be helpful in identification of unknowns in extracts from historical samples.

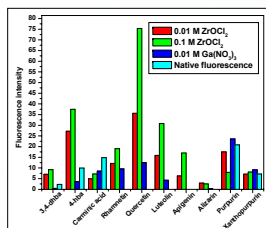


Changes in the UV-Vis spectrum of purpurin; double band in Vis range observed after deprotonation is characteristic for 1,4-dihydroxyanthraquinones.

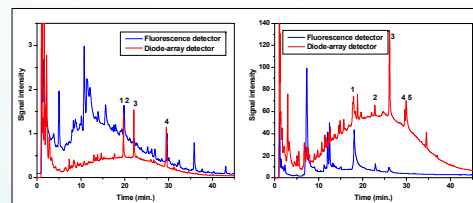
Changes in the UV-Vis spectrum of hystazarin after post-column complexation with zirconyl ion; characteristic for vicinal hydroxyl groups.

## Improvement of fluorescence detectability by post column complexation with zirconyl ion

Fluorescence detection (FLD) in HPLC analysis of dyes in extracts from historical objects is less often used, mainly with post-column complexation with aluminum and, with even better sensitivity, with gallium salts. Employing for this purpose zirconyl instead of mentioned ions offers lower detection limits than the DAD for several flavonoids, anthraquinones and flavonols' degradation products - hydroxybenzoic acids.



Comparison of fluorescence intensities without and with complexation with gallium and zirconyl ions.

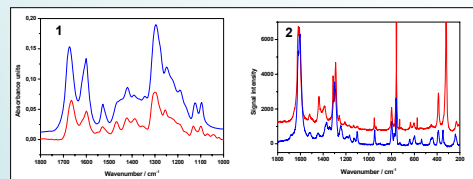


Chromatograms of extract from yellow thread from 16th Century tapestry from Brussels, France; 1 - luteolin, 2 - quercetin (identified only in FLD), 3 - apigenin, 4 - indigotin.

Chromatograms of extract from black thread from 19th Century tapestry from Aubusson, France; 1 - morin (identified only in FLD), 2 - kaempferol, 3 - alizarin, 4 - xanthopurpurin, 5 - purpurin.

## MID-IR and Raman detection with flow-through microdispenser

The application of a piezo-actuated flow-through microdispenser as a solvent elimination interface for IR and Raman detection in HPLC enables fully automated on-line deposition of HPLC eluate. The developed method is easy to handle, fast, does not require complex instrumentation and allows unambiguous identification of compounds through their IR and Raman spectra e.g. for degradation products of flavonoids.



Infrared (1) and Raman (2) spectra of 3,4-dihydroxybenzoic acid deposit (red line) and its standard (blue line).

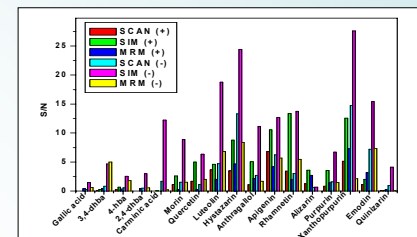
## ESI-Mass spectrometry for the best detection limits

### EXPERIMENTAL CONDITIONS

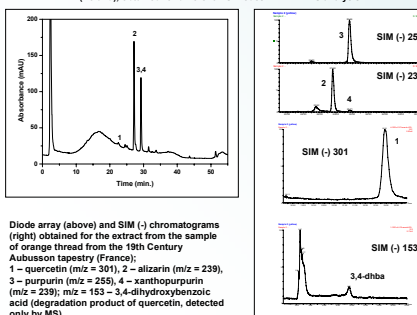
The work was done on a triple quadrupole mass spectrometer Quattro LC equipped with a ESI (electrospray) ion source, that could be connected on-line to the HPLC system. All separations were carried out at 17°C using the Zorbax RX-C8, 2.1 x 150 mm, 5 µm particle column with the injection volume equal 20 µl. The mobile phase consisted of gradient of acetonitrile in 0.3% (v/v) formic acid in water at the flow rate of 0.2 ml/min.

### LIMITS OF DETECTION

Compound	DAD	Native fluorescence	FLD, post-column complexation with 0.1 M ZrOCl <sub>2</sub> /ACN	FLD, post-column complexation with 0.1 M ZrOCl <sub>2</sub> /MeOH	MS - SIM (-)
3,4-dhba	8 ppb (260 nm)	87 ppb	44 ppb	3 ppb	1 ppb
2,4-dhba	17 ppb (256 nm)	56 ppb	5 ppb	1 ppb	2 ppb
Morin	24 ppb (352 nm)	531 ppb	6 ppb	3 ppb	3 ppb
Quercetin	9 ppb (370 nm)	455 ppb	13 ppb	5 ppb	2 ppb
Luteolin	4 ppb (348 nm)	-	91 ppb	31 ppb	1 ppb
Alizarin	3 ppb (250 nm)	777 ppb	1051 ppb	-	10 ppb
Purpurin	5 ppb (256 nm)	32 ppb	35 ppb	23 ppb	2 ppb (SIM (+))
Emodin	8 ppb (288 nm)	38 ppb	45 ppb	46 ppb	8 ppb



Values of signal to noise ratio (S/N) for the analyzed compounds dissolved in the extract prepared from the 4th-3th S.C. woolen thread from the archaeological excavations from Wroclaw (Poland), obtained for different MS modes in HPLC-MS analysis.



Diode array (above) and SIM (-) chromatograms (right) obtained for the extract from the sample of orange thread from the 19th Century Aubusson tapestry (France); 1 - quercetin (m/z = 301), 2 - alizarin (m/z = 239), 3 - purpurin (m/z = 255), 4 - xanthopurpurin (m/z = 239); m/z = 153 - 3,4-dihydroxybenzoic acid (degradation product of quercetin, detected only by MS).

## CONCLUSIONS

The best extraction method for flavonoids, anthraquinones, and indigotin proved to be one based on acidic hydrolysis followed by DMF/MeOH extraction. Determination of these three classes of dyes with HPLC method could be then performed in the same run.

Observation of double band in visible absorption region after post-column deprotonation was characteristic for 1,4-dihydroxyanthraquinones. Shifts of visible absorption maxima after post-column complexation with zirconyl chloride for flavonols were in good agreement with literature data, enabling the same primary structure elucidation of this class of dyes. Identification of vicinal hydroxyl groups in anthraquinones was obtained with post column complexation with zirconyl ions.

In the conditions tested zirconyl ion proved to be much better complexing reagent enhancing fluorescence detection than most often cited in literature aluminum and gallium ones, giving lower detection limits for all flavonoids and hydroxybenzoic acids than DAD. Sensitive analysis of flavonols, the most fugitive from natural dyes, and their degradation products - hydroxybenzoic acids, is of great importance in analysis of usually highly destructed archaeological textiles.

Mass spectrometer with the selected ion monitoring in electrospray negative ion mode proved to be the most sensitive and selective from all the detectors tested for HPLC analysis of natural dyes in extracts from archaeological threads.

1) B. Szostek, J. Orska-Gawrys, I. Surowiec, M. Trojanowicz, J. Chromatogr. A 1012 (2003) 179.  
2) M. Trojanowicz, J. Orska-Gawrys, I. Surowiec, B. Szostek, K. Urbaniak-Walczak, J. Kehl, H. Rajniak, M. Wróbel, Stud. Conserv. 49 (2004) 115.  
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