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Identification of main constituents of historical textile dyes by ultra performance liquid chromatography with photodiode array detection

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Ultra performance liquid chromatography equipped with a photodiode array detector (UPLC-PDA) was employed for the identification of main constituents of natural dyes. The dyestuffs investigated were the following: luteolin, apigenin, quercetin, rutin, gallic acid, carminic acid, alizarin, purpurin and indigotin. Among the three stationary phases (i. e., C18, C8 and phenyl) studied under reversed phase conditions, C18 phase provided slightly stronger retention and higher resolution. Complete separation of eight analytes (except indigotin) was achieved in less than 6 min by gradient elution with an acetonitrile-water mobile phase containing 0.1% trifluoroacetic acid (TFA). Strongly hydrophobic indigotin was determined in a separate UPLC run using isocratic elution with a 75 : 25 (v/v) acetonitrile / water mobile phase. The efficiencies of aqueous / methanolic HCl, EDTA and TFA solutions were compared for the extraction of natural dye components from dyed wool. The HCl system gave higher extraction yields than other two extractants. For the extraction of indigotin, dimethylformamide was selected as an extractant. Finally, the UPLC-PDA method was applied for the identification of dye compounds in the extracts of historical textiles samples.

Key words: ultra performance liquid chromatography, photodiode array detection, dyes, historical textiles

INTRODUCTION

Dyeing of textiles has been performed for more than 4,000 years, and for the longest part of this time natural organic materials have been the principal source of the dyestuffs, being replaced only during the last one and a half centuries or so by synthetic pigments and dyes which are much cheaper and more convenient to produce [1]. The knowledge of natural dyes composition in historical objects not only accesses to information on the subject dyes composition or dyeing technique, but also gives hints on proper restoration procedure. Moreover, the identification of individual dyestuffs which came into use at different times may help in identifying the historical period of the art object.

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In most cases, mixtures of colouring materials were used by the dyers to obtain the desirable hues [2]. Consequently, several dye compounds having similar structures and properties may be present in a sample extracted from a historical textile. For such samples chromatographic techniques with both separation and identification capabilities are preferred. High performance liquid chromatography (HPLC) equipped with a diode array detector is the most popular technique [3–7]. Several articles also refer to using a mass spectrometry (MS) detector, which provides with additional structural information [8–10]. Usually, the dyestuffs are separated in the reversed phase HPLC mode on C18 column using conventional acetonitrile / water or methanol / water mobile phases. However, due to significant polarity distribution, the retention of various dyestuffs differ significantly, therefore gradient elution is required for the separation of these compounds.

Despite the gradient elution, the multi-component analysis of real samples often requires separation times of 30 min and more.

Recent advances in analytical instrumentation have enabled the development of a new liquid chromatography technique termed ultra performance liquid chromatography (UPLC) [11]. A high speed of analysis, greater resolution, higher peak capacity and sensitivity are obtained due to the novel technology that utilizes a new generation of columns packed with pressure stable 1.7 μm hybrid material particles and novel low dead volume, high pressure (up to 1 000 bar) equipment [12–14].

In this study, UPLC equipped with a photodiode array (PDA) detector was employed for the separation and identification of main constituents of natural dyes extracted from historical textiles. For this purpose, UPLC separation and PDA detection parameters as well as extraction procedures were optimized. The structures of the dyestuffs investigated are provided in Fig. 1.

EXPERIMENTAL

UPLC separations were performed on the Waters Acquity UPLC system (Waters, Milford MA) equipped with an Acquity UPLC photodiode array (PDA) detector. The following columns maintained at 30 °C were used in the experiments: Acquity UPLC BEH C18, Acquity UPLC BEH C8 and Acquity UPLC BEH Phenyl (100 mm × 2.1 mm I. D., 1.7 μm, Waters). The injection volume was 5 μL using a partial loop with needle overfill injection mode. Data collection and management

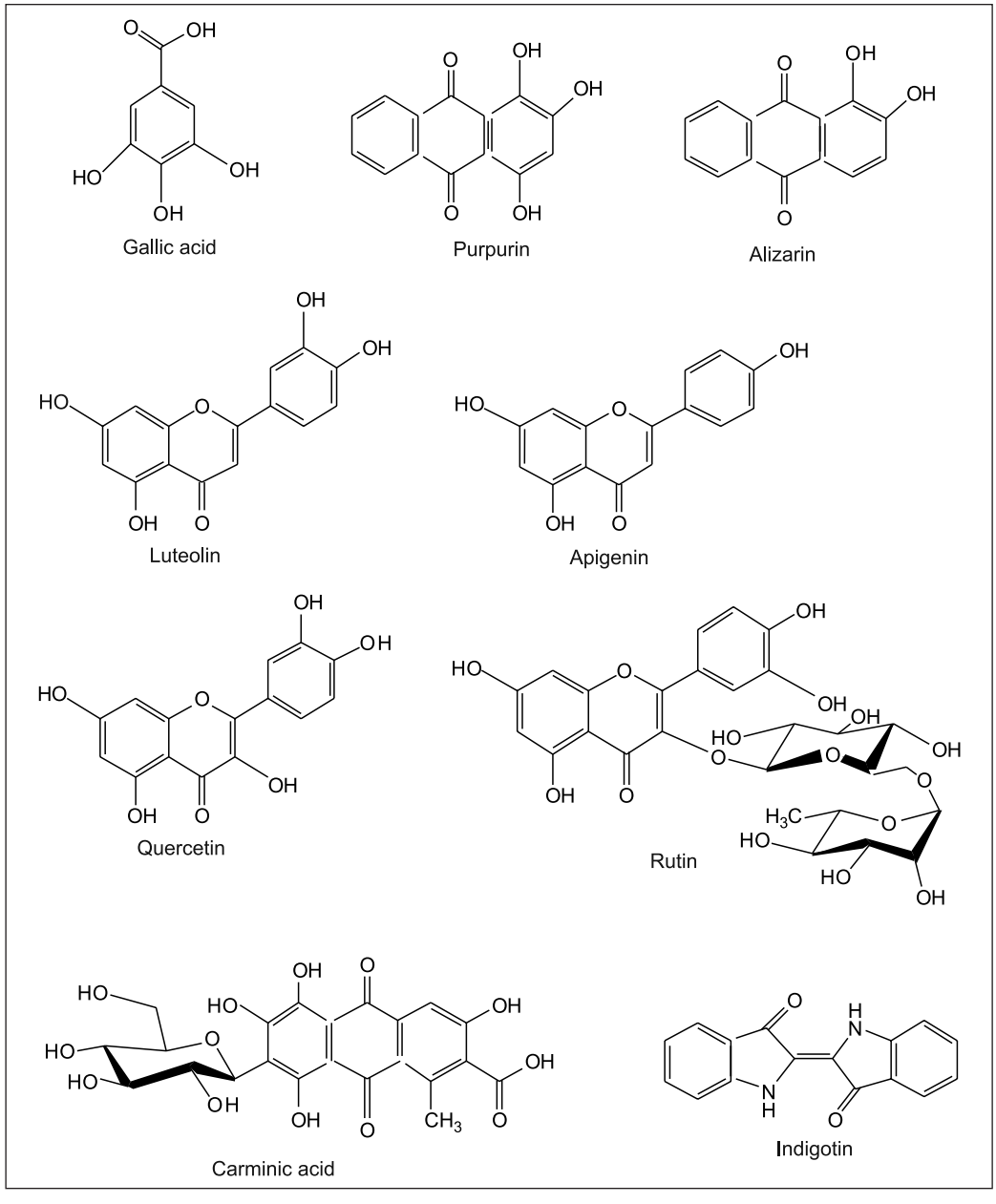

Fig. 1. Chemical structures of the main constituents of the natural dyes

were performed by the Empower 2 Build 2154 software (Waters).

Standards of alizarin (red), apigenin (yellow), gallic acid (dark brown), indigotin (blue), carminic acid (red), quercetin (yellow), luteolin (yellow), purpurin (red) and rutin (light orange) were chosen based on the literature source [2] and purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN), methanol, dimethylformamide (DMF), tetrahydrofuran (THF), formic acid and trifluoroacetic acid (TFA) were obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade, obtained from Sigma-Aldrich.

Stock solutions of dyes (except indigotin) at the concentration of 0.50 g/L were prepared in methanol and stored at 4 °C, protected from light. Working standard solutions were prepared fresh daily by diluting the stock solution with acetonitrile / water (1 : 1, v/v). Stock solution of indigotin at the concentration of 1.00 g/L was prepared in THF and stored at 4 °C, protected from light. Working standard solutions were prepared fresh daily by diluting the stock solution with acetonitrile.

Samples of threads from historical textiles were obtained from the Pranas Gudynas Center of Restoration.

RESULTS AND DISCUSSION

UPLC separation

The retention behaviour of nine dyes was initially evaluated on the C18 column in the isocratic elution mode with a 5 : 95 (*v/v*) acetonitrile / water mobile phase. Obtained results showed that the dyes studied differ significantly in their hydrophobicity: the retention times between the most retained purpurine and the least retained gallic acid differed about 25 times. Furthermore, the most hydrophobic indigotin was not eluted under selected conditions even in 60 min. Thus, due to the large difference in the retention times, fast UPLC separation of all dyes using isocratic elution was impossible.

Next, the retention properties of three commercial phases (C18, C8 and phenyl) were evaluated for a mixture of eight dyes in the gradient elution mode. The binary solvent system consisting of water (solvent A) and acetonitrile (solvent B) both containing 0.1% formic acid was employed. The addition of acid to the mobile phase lowered pH and suppressed the ionization of the acidic analytes. As can be observed (Fig. 2), comparable trends in elution orders and peak shapes were obtained on all three phases. As expected, the C18 phase was slightly more retentive for dyes than others. In addition, this phase gave considerably higher resolution of the critical luteolin / quercetin pair. Based on the above results, the C18 column was selected for further investigation.

Replacing formic acid with stronger TFA and $HClO_4$ did not significantly improve the overall separation performance. The use of TFA resulted in slightly better resolution between carminic acid and rutin. Complete separation of all analytes was achieved using the modified gradient program (Fig. 3).

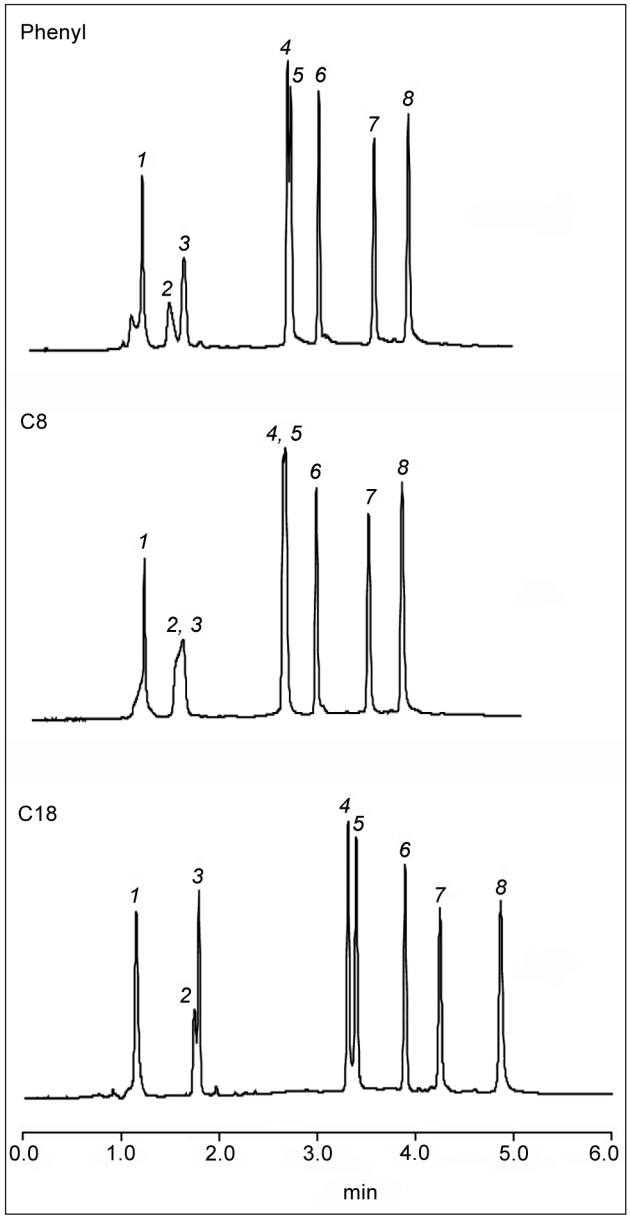

Fig. 2. Separation of dyestuffs on different stationary phases. Mobile phase A: 0.1% HCOOH in water. Mobile phase B: 0.1% HCOOH in ACN. Linear gradient: 20% → 90% B in 5 min. Flow rate – 0.25 mL/min. UV detection – 254 nm. Peaks: *1* – gallic acid; *2* – carminic acid; *3* – rutin; *4* – luteolin; *5* – quercetin; *6* – apigenin; *7* – alizarin; *8* – purpurin

As seen, all dyes were baseline separated in less than 6 min. However, under these conditions strongly hydrophobic indigotin does not elute from the column with a reasonable retention time and peak shape. For this reason indigotin was determined in a separate UPLC run using isocratic elution with a 75 : 25 (*v/v*) acetonitrile / water mobile phase.

Evaluation of the extraction procedures

Due to the limited amount of fibres from historical textiles the optimization of the extraction procedure was performed using wool samples mordanted with alum and dyed with pure dyestuffs according to the procedure described [15]. The

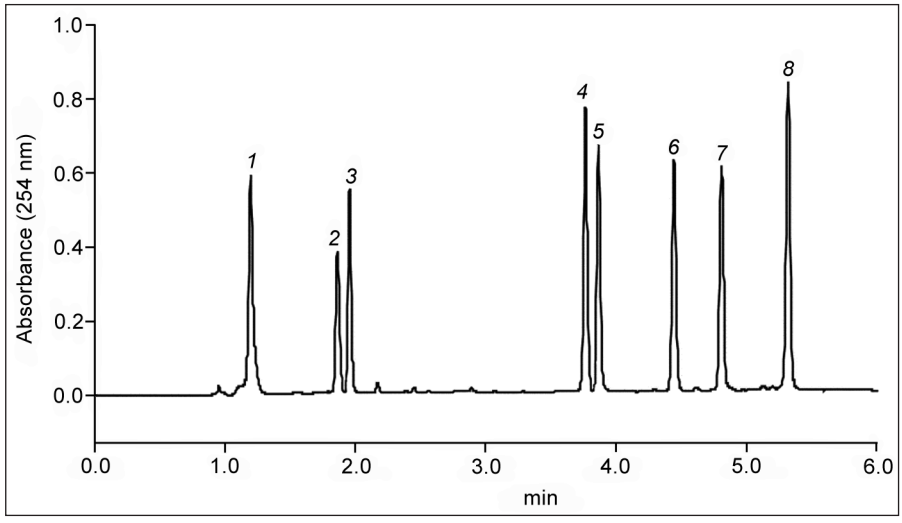

Fig. 3. Optimized UPLC separation of eight dyestuffs on C18 stationary phase. Mobile phase A – 0.1% TFA in water. Mobile phase B – 0.1% TFA in ACN. Gradient elution: 20% → 60% B from 0 to 3.5 min, 60% → 95% B from 3.5 to 4.5 min and 95% B from 4.5 to 5.5 min. Other conditions as in Fig. 2

extraction procedure for indigotin was optimized using fibres from blue jeans. Because most textiles are mordanted with metal ions, the extractant must be able to disrupt the metal-dye complex. The most widely used extraction protocol involves heating with an aqueous / methanolic HCl solution [3, 5, 16, 17]. Recently, several milder extraction protocols that use ethylenediaminetetraacetic (EDTA), formic [8], citric, TFA or oxalic [7] acid instead of HCl have been proposed.

In this study, three different extractant systems based on HCl, EDTA and TFA were compared. Samples of 3.0 mg of dyed wool were placed in capped vials, and 0.5 mL of water / methanol (1 : 1, v/v) mixture containing 2 mol/L of appropriate acid was added. The vial was heated for 15 min at around 100 °C in a boiling water bath. Then the mixture was cooled, centrifuged and subjected to UPLC analysis. The evaluation of the extraction efficiency was based on the comparison of the peak areas obtained from UPLC-PDA analyses for each dyestuff. A comparison of extraction efficiencies for wool dyed with five dyestuffs and extracted using the three extractants is presented in Fig. 4. Obtained results indicate that the HCl system gives higher extraction yields than other two extractants. Increasing the extraction time up to 30 min did not improve the extraction efficiency. Thus, HCl containing extractant was selected for sample analysis.

As expected, all three extraction systems tested were not suitable for the extraction of indigotin due to its low solubility in water / methanol mixtures [5, 7]. Therefore, for the extraction of indigotin two less polar solvents, namely DMF and THF, were tested. Samples of 3.0 mg of dyed wool were extracted with 0.5 mL of the appropriate solvent for 15 min at around 100 °C. The extracts were then evaporated to dryness under a stream of nitrogen and reconstituted with ACN

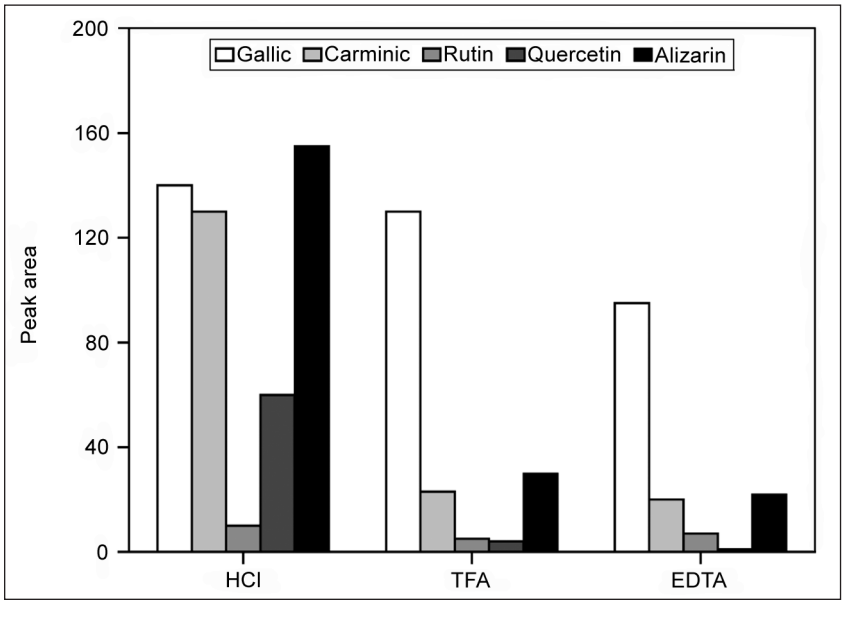

Fig. 4. Peak areas of the selected dyestuffs extracted with different extractants

Table. **Compounds identified in samples from historical textiles**

Textile	Sample	Sample colour	Compounds identified
"Apollo and Daphne" Flandreau, around 1679	wool	red	Alizarin, purpurin
	silk	mossy green	Rutin
"David and Bathsheba" Brussels, 1644–1654	wool	red	Alizarin, purpurin
	silk	mossy green	Rutin, luteolin
	silk	sandy yellow	Carminic acid
"Consecration of the Chapel" Brussels, 1644–1654	wool	red / brown	Alizarin, purpurin, rutin, luteolin

for the UPLC analysis. Although both solvents gave similar and acceptable extraction efficiencies, DMF was selected because it is compatible with the UPLC mobile phase. In this case time-consuming evaporation and reconstitution steps can be avoided. Finally, it was found that raising the extraction time from 5 to 20 min increased the extraction efficiency marginally. Thus, in the final protocol indigotin was extracted for 10, instead of 15 min.

Sample analysis

Samples (wool and silk threads) from three 16–17th century historical textiles were taken for identification of main dyestuffs extracted with HCl / water / methanol mixture. Three replicate extractions were performed for each sample. Identification of extracted dyestuffs was done by comparison of retention times and UV–Vis spectra recorded for sample extracts and pure standards. The results are summarized in the Table. The representative chromatogram of the red wool thread extract is shown in Fig. 5.

Finally, three historical textiles were investigated for indigotin: "Scene from Alexander the Great Marriage", Brussels, 16–17th century, "Salomon Treasures", Brussels, Jacob von Zeeman Workshop, 1640–1654, and "Elephant Walk", Flandreau, 16th century. Six green and blue wool threads from these textiles were analyzed and in all of them indogotin was identified. The representative chromatogram of the green wool thread extract is demonstrated in Fig. 6.

In conclusion, the results of the present study demonstrate that UPLC-PDA is a powerful technique for identification of main dyestuffs in historical textiles. Compared to current HPLC systems UPLC allows considerably faster separation with better resolution.

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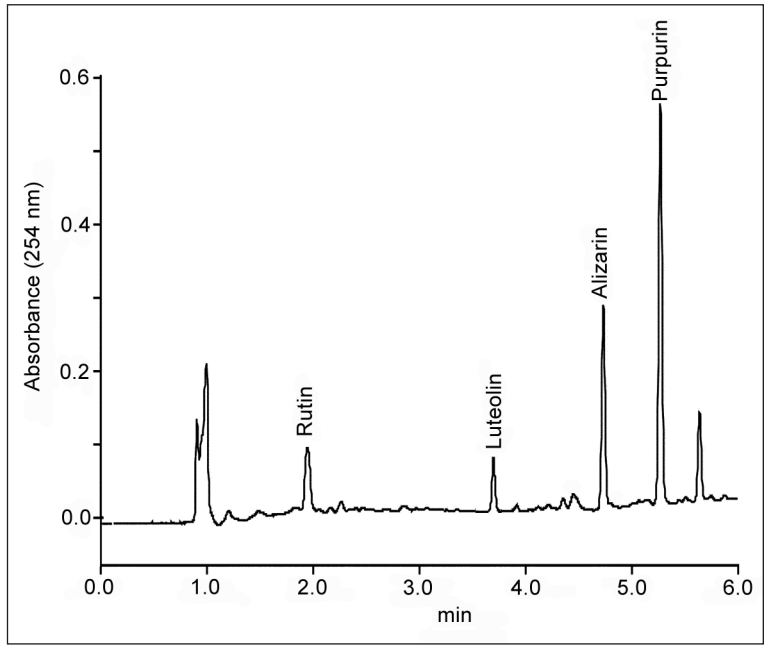

Fig. 5. Chromatogram of the sample (red / brown wool thread) extract from the textile "Consecration of the Chapel"

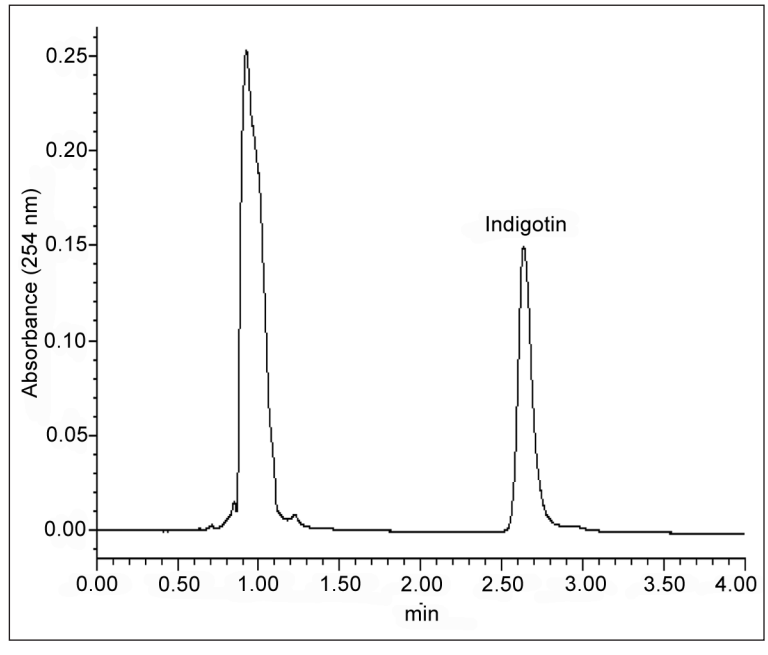

Fig. 6. Chromatogram of the sample (green wool thread) extract from the textile "Salomon Treasures"

References

1. E. Rosenberg, *Anal. Bional. Chem*., **391**, 33 (2008).
2. E. S. B. Fereira, A. H. Hulme, H. McNab, A. Quye*, Chem. Soc. Rev*., **33**, 329 (2004).
3. P. Novotna, V. Pacakova, Z. Bosakova, K. Stulik, *J. Chromatogr., A*, **863**, 235 (1999).
4. J. Orska-Gawrys, I. Surowiec, J. Kehl, H. Rejniak, K. Urbaniak-Walczak, M. Trojanowicz, *J. Chromatog*., *A*, **989**, 239 (2003).
5. I. Surowiec, A. Quye, M. Trojanowicz, *J. Chromatogr., A*, **1112**, 209 (2006).
6. I. Karapanagiotis, A. Lakka, L. Valianou, Y. Chryssoulakis, *Microchim. Acta*, **160**, 477 (2008).
7. L. Valianou, I. Karapanagiotis, Y. Chryssoulakis, *Anal. Bional. Chem*., **395**, 2175 (2009).
8. X. Zhang, R. A. Laursen, *Anal. Chem*., **77**, 2022 (2005).
9. I. Surowiec, B. Szostek, M. Trojanowicz, *J. Sep. Sci*., **30**, 2070 (2007).
10. X. Zhang, R. Boytner, J. L. Cabrera, R. Laursen, *Anal. Chem*., **79**, 1575 (2007).
11. M. Swartz., *J. Liq. Chromatogr. Relat. Technol*., **28**, 1253 (2005).
12. G. Kesiūnaitė, E. Naujalis, A. Padarauskas, *J. Chromatogr., A*, **1209**, 83 (2008).
13. A. Orentienė, V. Olšauskaitė, V. Vičkačkaitė, A. Padarauskas, *Chromatographia*, **73**, 17 (2011).
14. A. Orentienė, V. Olšauskaitė, V. Vičkačkaitė, A. Padarauskas, *J. Chromatogr., A*, **1218**, 6884 (2011).
15. E. S. B. Fereira, A. Quye, A. H. Hulme, H. McNab, *Dyes History Archaeol.*, **18**, 63 (2002).
16. J. Wouters, *Stud. Conserv*., **30**, 119 (1985).
17. J. Wouters, A. Verhecken, *Stud. Conserv.*, **34**, 189 (1989).

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DAŽIŲJŲ MEDŽIAGŲ IDENTIFIKAVIMAS ISTORINĖJE TEKSTILĖJE ULTRAEFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS SU FOTODIODINĖS MATRICOS DETEKTORIUMI METODU

S a n t r a u k a

Ultraefektyvioji skysčių chromatografija su fotodiodinės matricos detektoriumi (UPLC-PDA) pritaikyta pagrindinių natūralių dažiklių komponentų identifikavimui. Darbe tirti šie dažikliai: galo rūgštis, karmino rūgštis, rutinas, liuteolinas, kvercetinas, apigeninas, alizarinas, purpurinas ir indigotinas. Palyginus dažiklių atskyrimą trimis skirtingais atvirkščių fazių sorbentais (C18, C8 ir Fenil) nustatyta, kad C18 sorbentu dažikliai yra sulaikomi stipriau ir geriau atskiriami. Aštuoni dažikliai (išskyrus indigotiną) visiškai atskiriami per 6 min. vandens / acetonitrilo mišiniu su 0,1 % trifluoracto rūgšties (TFA) priedu naudojant gradientinę eliuciją. Akivaizdžiai hidrofobiškesnis indigotinas identifikuojamas atskirai naudojant izokratinę eliuciją 75 : 25 (*v/v*) acetonitrilo / vandens mišiniu. Palyginta dažiklių ekstrakcija iš vilnos siūlų vandens / metanolio tirpalais su HCl, EDTA ir TFA priedu. Efektyviausiai dažikliai ekstrahuojami HCl tirpalu metanolio / vandens mišinyje. Indogotino ekstrakcijai pasirinktas dimetilformamidas. UPLC-PDA metodas buvo panaudotas dažikliams identifikuoti istorinių tekstilių mėginiuose.