Inter-Laboratory Chemical Study of Natural Materials from the Historical Wiesner Collection

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Abstract

Microsamples of *Rubia tinctorum L.*, *Isatis tinctoria L.*, *Indigofera tinctoria L.*, *Opuntia decumana L.* and *Caesalpina echinata Lamarck* were analyzed independently by five institutions, which applied their own analytical protocols and instrumentation. Samples were extracted from the historical "Wiesner Raw Material Collection" which is in the ownership of the Vienna University of Technology. Two analytical techniques were employed; High Performance Liquid Chromatography (HPLC) and Pyrolysis Capillary Gas Chromatography/Mass Spectrometry (Py-CGC/MS). For the HPLC experiments, samples were treated with acidified methanol, a method which is extensively used to extract dyes from (historical) textile fibres. For the Py-CGC/MS studies no sample treatment was necessary. Colouring compounds, which are used as indices for the assessment of dyestuff sources, were identified in all samples and with almost all analytical protocols. Comparable results were obtained with both analytical techniques, HPLC and Py-CGC/MS.

Keywords: Dye; Madder; Cochineal; Indigo; Woad; Redwood; HPLC; GC/MS.

Introduction

Five dyestuff sources which belong to the important "Wiesner Raw Material Collection" from the nineteenth century are analyzed in the present study. The collection is in the ownership of the Vienna University of Technology. In reality two collections are available; the elder one goes back to 1815, the year of the foundation of the "Vienna Polytechnical Institute", and contains 1500 little sample glasses with hand-written labels, mainly filled with organic materials and are divided into twenty one sections. The newer part of the "Wiesner Collection" (stemming from the second half of the nineteenth century) consists of 5000 vessels, most of them contain organic

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materials and are grouped in 21 sections. Regarding the topic of the present study both collections taken together include a treasure of 250 dyes and pigments, e.g. concerning indigo eighty samples from countries all over the world are available.

Microquantity samples out of the Wiesner collection were taken and extracted for further studies. The materials were *Rubia tinctorum L.*, *Isatis tinctoria L.*, *Indigofera tinctoria L.*, *Opuntia decumana L.* and *Caesalpina echinata Lamarck* and were distributed to five analytical laboratories which applied their own analytical protocols and instrumentation, according to the experimental section. The inter-laboratory investigation was performed within the frameworks of the INCO CT 2005 015406 MED-COLOUR-TECH project (www.medcolourtech.org) which is supported by the EU. The institutions which are involved in the study are the Ormylia Art Diagnosis Centre (OADC), Greece; Marmara University (MU), Turkey; University of Jordan (UJ), Jordan; Yarmouk University (YU), Jordan, and Vienna University of Technology (VUT), Austria. The aforementioned laboratories applied protocols 1, 2, 3, 4 and 5, respectively, as described in the Experimental.

The aim of this present investigation is to compare the analytical results obtained by five independent laboratories working with their own analytical protocols and using their own techniques. The latter include High Performance Liquid Chromatography (HPLC) and Pyrolysis Capillary Gas Chromatography/Mass Spectrometry (Py-CGC/MS). For this reason five samples, described previously, are extracted from the important “Wiesner Raw Material Collection” and analyzed according to the following.

Experimental

Samples

The following samples of the Wiesner collection were analyzed in this study: *Rubia tinctorum* L. (sample 1: Hungarian madder or ground madder from the “Pester Comitate”, year 1824, number 562), *Isatis tinctoria* L. (sample 2: indigo from Hungarian woad, Triester exhibition 1882, R. v. Höhnel, number 66), *Indigofera tinctoria* L. (sample 3: indigo, the prepared leaf-juice, Bombay, bazar, Feb 1869, number 9), *Opuntia decumana* L. (sample 4: cochineal, dried female beetles of *Dactylopius coccus Costa*, number 537) and *Caesalpina echinata* Lamarck (sample 5: extract of brazilwood, K.K. Technical University–Raw Material Cabinet, Family Caesalpiniaceae, year 1826/27, number 61).

Standard compounds of dyes

Alizarin and brazilin, employed as reference materials, were purchased from Aldrich and Pfaltz & Bauer, respectively, and were used as received. Purpurin was obtained as 90% pure from Aldrich and was purified through several recrystalization procedures from ethanol. Indigotin[1], indirubin[2], Munjistin[3], rubiadin[4], xanthopurpurin[5], were sythesized, purified, and characterized according to literature procedures. Flavokermesic acid and kermesic acids were extracted from their natural

source, *Kermes vermilio* Planchon according to published procedures[6] by subjecting the extract to TLC silica gel G containing oxalic acid (2%) in toluene/ethyl acetate (7 : 3) and in toluene/acetone/acetic acid (60:40:1) for flavokermesic acid and kermesic acid, respectively. The extract obtained from *Kermes vermilio* Planchon contains both kermesic and flavokermesic acids, among others. The separation of these two components from each other was not easy; a mixture is always obtained. Several separations had to be performed to obtain both components in pure forms as indicated by their TLC behavior.

Analytical protocols

Protocol 1 (OADC). The standard HCl extraction process, which is widely adopted to extract the organic colorants from a textile substrate[7,8] was employed to treat the samples. The original method was applied with some minor modifications such as the solvents used in step (iii) as it is described below. Apparently, acid hydrolysis was not necessary to extract the colouring compounds from their biological sources, investigated in the present work. However, the standard textile HCl treatment process[7,8] was applied so that the analytical results collected in this study can be used as a reference database for any future analysis of historical samples. The process is summarized as follows: (i) sample is immersed in 400 µL of H_2O: MeOH: 37 % HCl (1:1:2, v/v/v) at 100 ^oC for 15 min. (ii) The liquid phase is evaporated (65 ^oC) under gentle nitrogen flow. (iii) The dry residue is dissolved in DMF (blue dyestuffs) or MeOH: H_2O (2:1) (reddish dyestuffs). (iv) Finally, sample is centrifuged and submitted to HPLC analysis.

Analysis was carried out by an HPLC system (Thermoquest, Manchester, UK) which consists of a P4000 quaternary pump, an SCM 3000 vacuum degasser, an AS3000 auto sampler with column oven, a Reodyne 7725i Injector with 20 µL sample loop and a Diode Array Detector UV 6000LP. A reversed phase Alltima HP C18 5µm column with dimensions 250 mm x 3.0 mm (Alltech Associates, Inc., USA) was utilized for separation. The temperature of the column was 33 ^oC and gradient elution was performed using two solvents; A: 0.1 % (v/v) triflouroacetic acid (TFA) in water and B: 0.1 % (v/v) TFA in acetonitrile. The elution program is described in details elsewhere[9].

Protocol 2 (MU): Dyestuff extraction was done according to previously described method[7,8,10]. Historical textile samples (0.4-1.0 mg) were hydrolysed with H_2O: MeOH: 37% hydrochloric acid (1:1:2; v/v/v; 400 µl) in conical glass tubes for precisely 8 min in a water bath at 100 ^oC to extract the organic dyes. After rapid cooling under running cold water, the solution was evaporated just to dryness in a water bath at 50-65 ^oC under a gentle stream of nitrogen. The dry residue was dissolved in 200 µl of a mixture of MeOH: H_2O (2:1; v/v) and was centrifuged at 2500 rpm for 10 min. Then 25 µl and/or 50 µl of the supernatant were injected into the HPLC instrument. Residue was

dissolved in 200 µl of N,N-dimethylformamide because indigotin is not soluble in MeOH:H_2O (2:1; v/v) and was then injected into the HPLC separation system.

Chromatographic experiments were performed using an Agilent 1100 series system (Agilent Technologies, Hewlett-Packard, Germany) including a model G1311A gradient delivery pump with a 50 µl loop and a Rheodyne valve (7725i sample injector), a G1315A diode-array detector (Chromatograms were obtained by scanning the sample from 191 to 799 nm with a resolution of 2 nm and chromatographic peaks were monitored at 255, 268, 276, 350 nm and 491 nm), a G1322A vacuum degasser and a G1316A thermostatted column compartment; the data station was an Agilent Chemstation. A Nova-Pak C_{18} analytical column (3,9 x 150 mm, 4µm, Part No WAT 086344, Waters) protected by a guard column filled with the same material, was used. Analytical and guard columns were maintained at 30 ^oC. The HPLC gradient elution was performed using the method of Halpine et al[11] and Karapanagiotis et al.[12]. Chromatographic separation was accomplished using a gradient elution program that utilizes two solvents; A: H_2O-0.1%TFA and B: CH_3CN-0.1%TFA at a flow rate was 0.5 ml/min.

Protocol 3 (UJ): The process is summarized as follows: (i) sample is immersed in H_2O: MeOH: 37 % HCl (1:1:2, v/v/v) at a ratio of 400 µL/1 mg sample at 100 ^oC for 15min. (ii) The liquid phase is evaporated (60 ^oC) under gentle nitrogen flow. (iii) The dry residue is dissolved in DMF at a ratio of 1 mL/1 mg sample. (iv) sample is then centrifuged and 20 µl of the supernatant were injected into the HPLC column. Analysis was carried out with the aid of an HPLC system (GBC, Australia) which consists of pump, a Rheodyne Injector type 7125 with a 20 µl sample loop, a column oven type GBC(TC 300 Controller/Australia) and a Diode Array Detector (DAD) type Knauer 2800(Germany). The column used was Hypersil Gold C18 (Phenomenex, USA) 250 x 4.6 mm (5 µm particle size). The temperature of the column was 40 ºC. Isocratic elution was performed using a solvent mixture of Acetontril/water/methanol (40:38:22%) + 0.1% trifluoroacetic acid as eluent at a flow rate of 1.0 mL/min. The Diode Array Detector was programmed as follows:
0-6.8 min (290 nm); 6.8-7.5 min (270 nm); 7.5-9.0 min (290 nm); 9.0-109 min(289 nm); 10.9 – 14.0 min (280 nm); 14.0 – 19.0 min (300 nm) and 19.0- 60.0 min (289 nm).

Protocol 4 (YU): The standard HCL extraction method used for historical dyed textile samples was employed to extract the colour components from the samples[13,14]. The process is summarized as follows: (i) A sample was immersed in 400 µL of H2O: MeOH: 37 % HCl (1:1:2, v/v/v) and kept at 100 ^oC for 10 min to extract the organic dyes. (ii) The sample was evaporated by heat and vacuum-dried. (iii) The dry residue is dissolved in 200 µL of DMF (blue dyestuffs) or MeOH: H_2O (2:1) (reddish dyestuffs). (iv) The solution was then filtered through a 25 mm 0.45-micron cellulose syringe filter. Analysis was carried performed with a VARIAN Prostar HPLC system which consists

of a P4000 ternary HPLC pump, a Reodyne 7725i Injector with 20µl sample loop and a Diode Array Varian Prostar Detector. The instrument is equipped with a Star Chromatography work station Version 6.41. PDA and FLD detections were achieved by scanning from 200 to 800 nm with a resolution of 2 nm. A reversed phase NOVA C18 5µm column with dimensions 150 mm x 4.0 mm was utilized for separation. HPLC analysis was performed using the following gradient. The eluent was a mixture of water/ methanol / 5% (w/v) phosphoric acid (60: 30: 10) for 3 minutes followed by a linear gradient of (10: 80: 10) for 26 minutes at a flow rate of 1.2 mL/minute, creating a system back-pressure of 18 to 24 Mpa. The temperature in the chromatography laboratory was 20 °C ± 2. Both the retention time and the UV-Vis spectrum of each component the tested dye were recorded and identified.

Protocol 5 (VUT): Five organic dyestuffs (described above) were analyzed by means of pyrolysis capillary gas chromatography / mass spectrometry[15-18]. This is a method, which is well-known for its tiny demand of samples (applied amount of colouring material in this study ca 1 mg) as well as the fact that no time-consuming extraction process is necessary before chromatography. In this study, a modern "FrontierLab Double-Shot Pyrolyser" was used allowing both, "Desorption Analysis" as well as recording pyrograms.

Although these analyses were done directly (without preparation of dyeing materials), there are still some publications dealing with this subject that propose to derivatize the samples before their pyrolysis[18]. In most cases this step is not absolutely necessary as could be shown from our research group presented as reports prepared within the project MED-COLOUR-TECH. This additional work seems only justified in the case of organic acids of dyes (e.g. carminic or kermesic acid in cochineal) or in the pyrolysis of components with hydroxy groups, which otherwise would not be detectable.

Analyses were performed with the aid of a Double-Shot Pyrolyser (FrontierLab 2010D) for thermal Desorption and pyrolysis, a Thermo Trace GC Ultra"; column: Rtx-5, 30 m x 0,25 mm; film thickness: 25 µm; carrier gas: He; pressure (column head): 50 kPa; injector temp.: 320°C for capillary gas chromatography, and a Thermo DSQ II Single Quadrupole Mass Spectrometer"; EI: 96 eV; scan rate: 1,09 scans/sec; scan range: 50-500 m/z; ion source temperature: 240°C; NIST library for Mass spectrometry. Nomenclature of pyrolyzates is given in accordance to NIST library.

Conditions for "Thermal Desorption: Furnace program: 50 °C (2 min), heating rate: up to 300 °C (25 °C/min), holding temp.: 300 °C (3 min)

Interface temp.: 330°C manual. CGC oven program: 35 °C (15 min), heating rate 1: up to 200 °C (25 °C/min), heating rate 2: up to 300 °C (10°C/min), holding temp.: 300 °C (18. 4 min). Conditions for pyrolysis*:* Pyrolysis temperature: 750 °C; pyrolysis time: 2 min; interface temp.: 330 °C

CGC oven program: 50 °C (2 min), heating rate: up to 300 °C (5 °C/min), holding temp.: 300 °C (18 min)

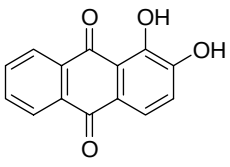
Results and discussions

The results collected for the five dyestuff sources which were included in the study are summarized in Tables 1-5. Table 1 shows the results of the madder samples. Tables 2, 3, 4 and 5 describe the colouring compounds detected in woad, indigo, cochineal and brazilwood, respectively. Below, the results collected for each analytical protocol are discussed. Relatively detailed discussions are provided for the results obtained with protocols 1 and 5. The former includes the use of HPLC and the latter includes the use of Py-CGC/MS. According to the Experimental protocols 2, 3 and 4 are based on HPLC analysis (similar to protocol 1) and therefore, the results obtained with these methods are briefly summarized. It is noteworthy that HPLC based protocols 1, 2, 3 and 4 employ the widely adopted dyestuff extraction method applied to extract natural organic dyes from historical textiles[7,8].

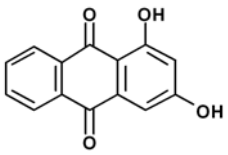
Table 1: Results for *Rubia tinctorum* (madder)

Protocol	Identified colouring compounds
Protocol 1 (OADC)	alizarin, xanthopurpurin, purpurin, rubiadin
Protocol 2 (MU)	munjistin, alizarin, purpurin, rubiadin
Protocol 3 (UJ)	alizarin, xanthopurpurin, purpurin
Protocol 4 (YU)	alizarin, purpurin
Protocol 5 (VUT)	alizarin, rubiadin

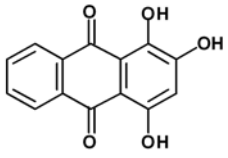
Protocol 1 (OADC): Alizarin (AL), xanthopurpurin, purpurin (PU) and rubiadin were detected in the extract of the madder sample, as presented in Table 1. The relative peak area ratio of alizarin vs. purpurin was measured for the chromatogram collected at 254 nm and was found to be 1.2. The result can be indicative of the biological source of the madder sample, as in *Rubia tinctorum* L. alizarin and purpurin are contained in comparable quantities. This is not true for other Mediterranean madder species. For example it has been reported that in wild madder (*Rubia peregrina* L.) alizarin is contained in smaller quantities than purpurin and sometimes it is hardly detected[19,20].



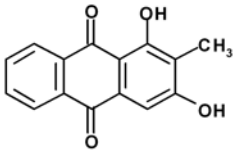
Alizarin



Xanthopurpurin

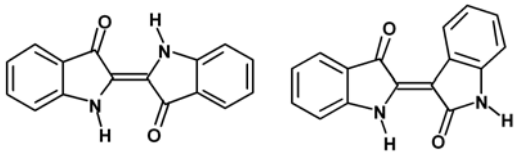


Purpurin



Rubiadin

Indigotin (IND) and indirubin (INR) were detected in woad and indigo samples as given in Tables 2 and 3. Figure 1 shows the chromatograms collected at 288 nm for the two blue dyestuff sources and suggests that the IND/INR peak area ratio recorded for woad is clearly higher than the corresponding ratio calculated for indigo. In particular, the ratios are different by an order of magnitude: 15.5 for woad and around 120 for indigo). We note, however, that the observed difference in the relative composition of the two dyestuffs cannot be used as a criterion to distinguish indigo from woad. Although hundreds of samples have been investigated it is still not possible to separate woad from indigo[14].



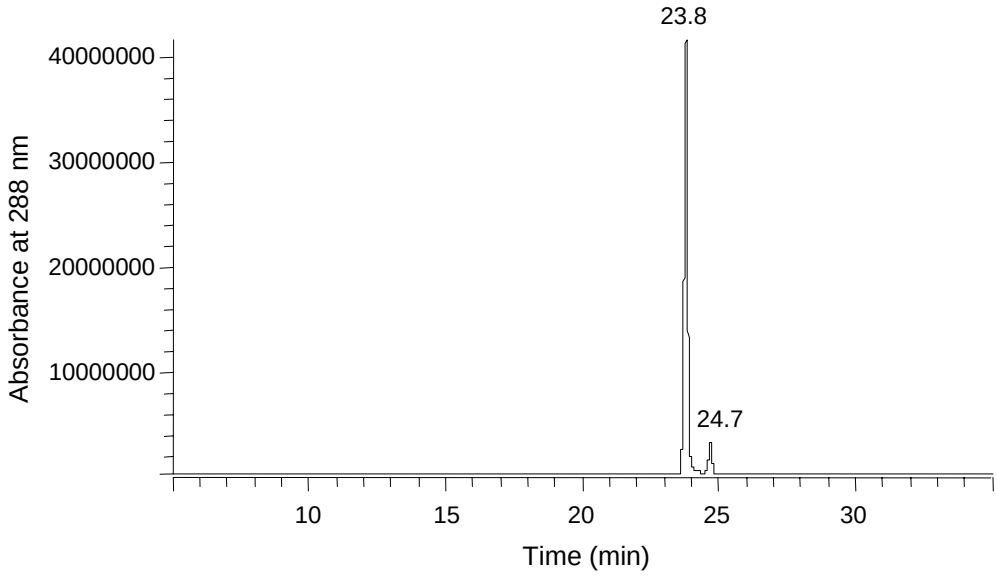
Indigotin Indirubin

Table 2: Results for *Isatis tinctoria* (woad)

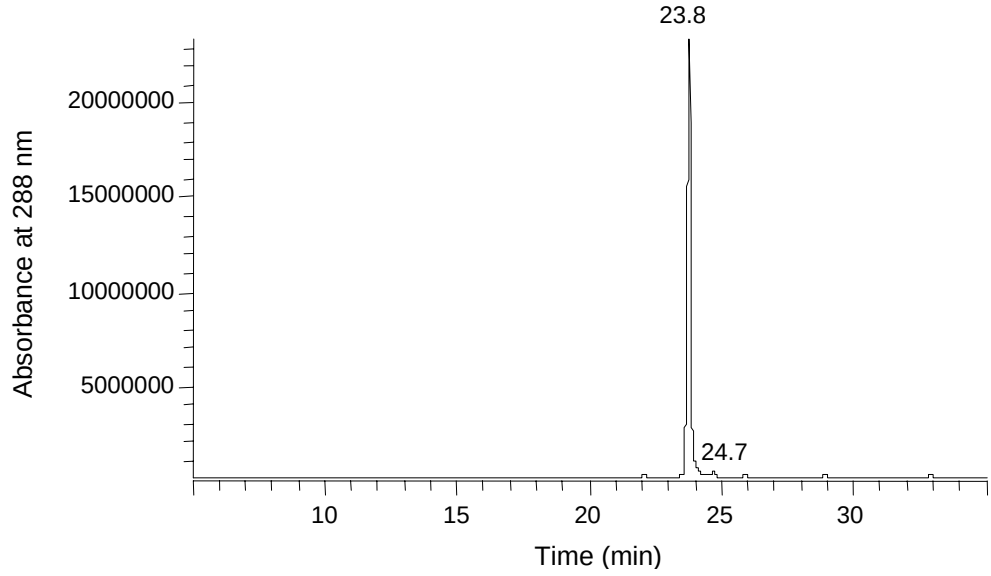
Protocol	**Identified colouring compounds**
Protocol 1 (OADC)	indigotin, indirubin
Protocol 2 (MU)	indigotin, indirubin
Protocol 3 (UJ)	indigotin, indirubin
Protocol 4 (YU)	ndigotin, indirubin
Protocol 5 (VUT)	indigotin indirubin

Table 3: Results for *Indigofera tinctoria* (indigo)

Protocol	**Identified colouring compounds**
Protocol 1 (OADC)	indigotin, indirubin
Protocol 2 (MU)	indigotin, indirubin
Protocol 3 (UJ)	indigotin, indirubin
Protocol 4 (YU)	indigotin, indirubin
Protocol 5 (VUT)	Indigotin, indirubin



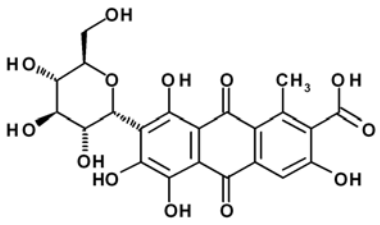
(a)



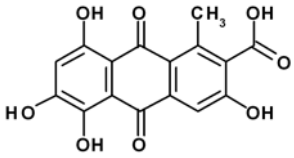
(b)

Figure 1: Chromatograms collected for (a) *Isatis tinctoria* L. and (b) *Indigofera tinctoria* L. at 288 nm. Indigotin (23.8 min) and indirubin (24.7 min) are identified.

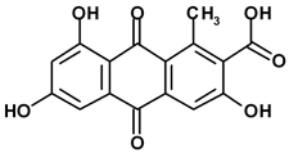
The HPLC cromatogram collected for cochineal is shown in Figure 2. Carminic (CA), kermesic (KA) and flavokermesic (FL) acids, dcII, dcIV and dcVII were detected, as shown in Table 4 and Figure 2. The latter suggests that KA and FL were contained in very small quantities and dcII was clearly identified. These results are indicative of the provenance (America) of the tested cochineal sample, according to previously published reports[8].



Carminic Acid



Kermesic acid



Flavokermesic acid

Table 4: Results for *Opuntia decumana* (cochnineal)

Protocol	Identified colouring compounds
Protocol 1 (OADC)	dcII, carminic acid, dcIV, dcVII, kermesic acid, flavokermesic acid
Protocol 2 (MU)	dcII, carminic acid, dcIV, dcVII, kermesic acid
Protocol 3 (UJ)	-
Protocol 4 (YU)	carminic acid, kermesic acid, flavokermesic acid
Protocol 5 (VUT)*	-

* no component, only the dyestuff was identified

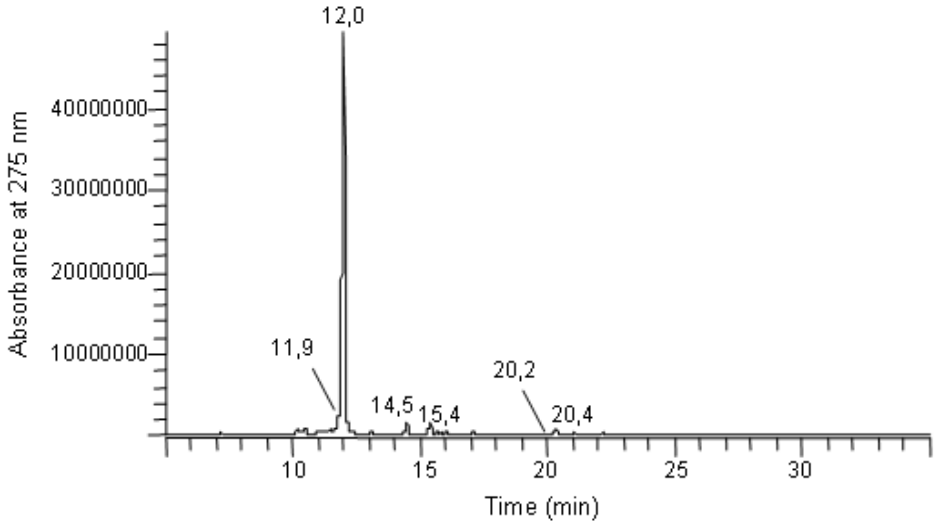
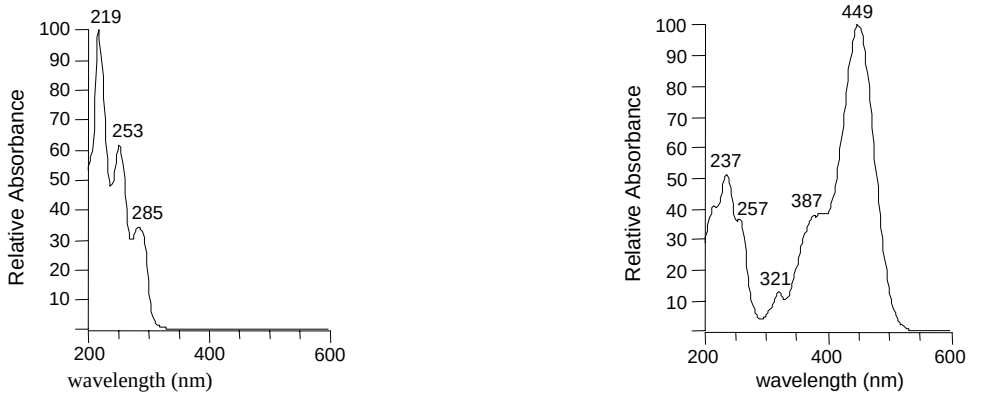


Figure 2: Chromatogram collected for *Opuntia decumana L* The peaks at 11.9, 12.0, 14.5, 15.4, 20.2 and 20.4 min correspond to dcII, carminic acid, dcIV, dcVII, kermesic acid and flavokermesic acid, respectively.

Table 5 summarizes the colouring compounds identified in the brazilwood sample and Figure 3 provides their spectra, collected by the diode array detector. Brazilin (Figure 3a) and a type B compound, which is also known as Bra´ (Figure 3b), were detected. Both compounds can be used as markers for the identification of soluble redwoods[9,21]. Additionally, several other unknown colorants were detected in the extract of the brazilwood sample which, however, could not be identified and therefore, are not discussed.

Table 5: Results for *Caesalpina echinata* (brazilwood)

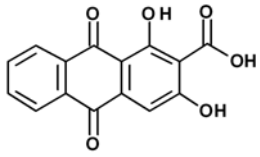
Protocol	Identified colouring compounds
Protocol 1 (OADC)	brazilin, type B compound
Protocol 2 (MU)	brazilin
Protocol 3 (UJ)	-
Protocol 4 (YU)	brazilin
Protocol 5 (VUT)	brazilin



(a) (b)

Figure 3: Spectra of (a) brazilin and (b) type B compound (Bra´).

Protocol 2 (MU): Munjistin, alizarin, purpurin and rubiadin were identified in the extract of madder (*Rubia tinctorum* L.) sample (Table 1); alizarin and purpurin are the major madder components. Indigotin and indirubin were identified in woad (*Isatis tinctoria* L.) and indigo samples (Tables 2 and 3) while carminic acid, kermesic acid, dcII, dcIV and dcVII were identified in cochineal according to table 4 with carminic acid being the major component. As for brazilwood (*Caesalpinia echinate* Lam), brazilin was identified as shown in table 5. Several other compounds were detected in the extract of the brazilwood sample but they could not be identified.



Munjistin

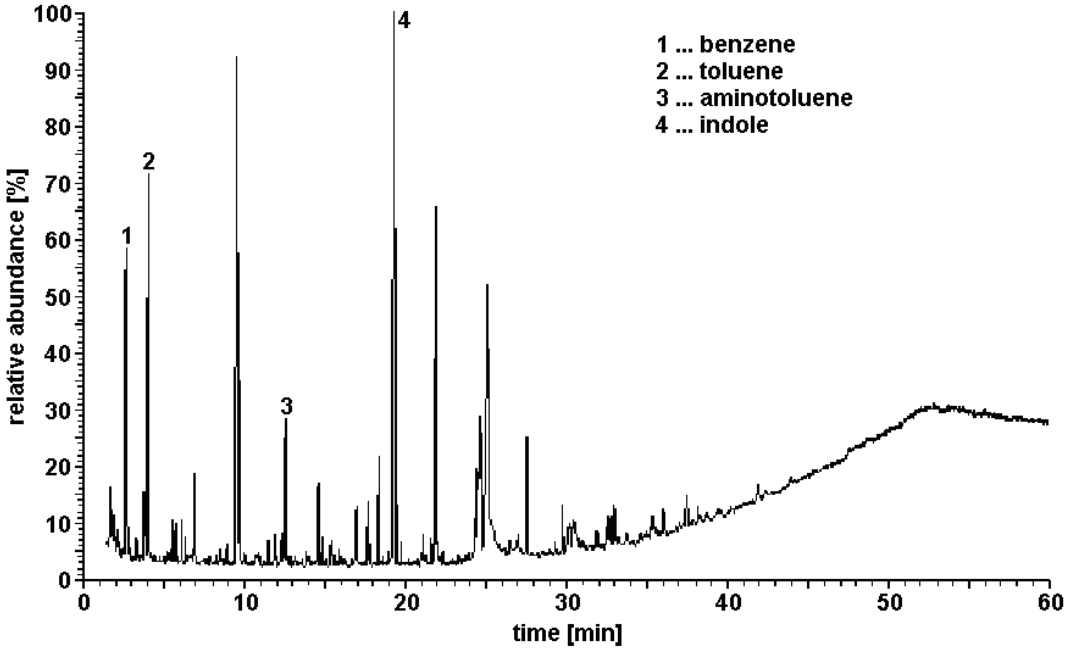
Protocol 3 (UJ): According to data presented in tables 1-3, similar results with the previously discussed protocols were obtained for madder, woad and indigo. No colouring compound was possible to be identified in cochineal and redwood samples.

Protocol 4 (YU): The results obtained with this protocol are similar with the previously described methods (i.e. protocols 1-3).

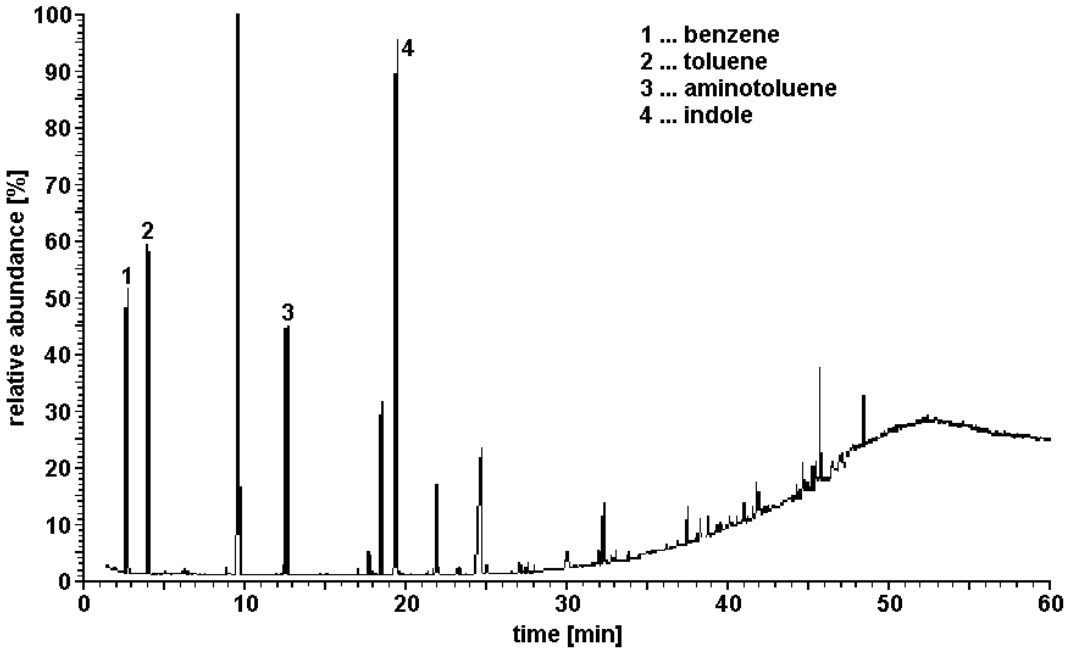
Protocol 5 (VUT): While all the other working groups were primarily using HPLC for their analytical work, VUT tried to supplement the usual analytical methodology by an additional analytical technique; Py-CGC/MS (pyrolysis linked to capillary gas chromatography plus mass spectrometry), thus throwing some light “from outside” on the traditional method and its results. The dyeing components occurring in sample 1, *Rubia tinctorum* (alizarin, purpurin and rubiadin), can be distinguished by the ratio of the pyrolyzates (anthraquinone, m/z 152, 180, 208 / hydroxyanthraquinone, m/z 139, 168, 224 / hydroxy-methylanthraquinone, m/z 238). By this ratio it could be shown that purpurin obviously is only present in small amounts within the crude sample. This could also be confirmed by thermal desorption (TD)” (usage of the pyrolyzing equipment at temperatures below 300 °C). The occurrence of alizarin in larger quantity in sample 1 is confirmed by the fact that the intensity of the hydroxyanthraquinone-signal is five times higher than that of anthraquinone.

For samples 2 and 3 (*Isatis tinctoria* and *Indigofera tintoria)* where analyses were performed only by recording TIC (total ion current) no differentiation could be found out; this became possible by TD considering the relative intensities of the compounds indole, 1,3-dihydroindole-2-one and octadecenoic acid. Pyrolysis of the main components of indigo (indigotin and indirubin) results in many degradation products, whereby the relative intensities of four substances – benzene (m/z 77, 78), toluene (m/z 91, 92), aminotoluene (m/z 106, 107) and indole (m/z 89, 90, 117) - give some information on the quantity of indigotin and indirubin in the dyeing material. In the case of the reference substance indigotin, this ratio (benzene : toluene : aminotoluene : indole) is 9:2:1:5. In relation to component indirubin, an intensity ratio of 4:4:4:8 was measured (Figure 4b). From the benzene : toluene : aminotoluene : indole ratio it can be concluded that indirubin predominates in both dyestuffs (Figure 4a). This result is of interest as it disagrees with the results obtained by the conventional HPLC, described previously. Further investigations will be necessary to clear up the surprising results found by Py-CGC/MS.

The pattern of products built during pyrolysis of *Opuntia decumana* (sample 4) is very different from other dyeing materials. In the pyrogram the following compounds could be identified: undecene (m/z 55), dodecene (m/z 69), hydroxycinnamic acid (m/z 91, 147, 163, 164), tetradecanoic acid (m/z 55, 60, 73, 129), octadecanol (m/z 55, 69, 83, 97) und nonadecanone (m/z 58, 71, 85, 96). The real main component carminic acid as well as the compounds kermesic- and flavokermesic acid could not be observed in the pyrogram of *Opuntia decumana* as a consequence of sample application without derivatization. These acids can only be vaporized and visualized in a pyrogram, if their hydroxy groups are derivatized, e.g. with tetramethylammonium hydroxide[18].



(a)



(b)

Figure 4: TIC-pyrograms of (a) *Indigofera tinctoria* and (b) indirubin obtained by Py-CGC/MS.

Analyzing brazilin in *Caesalpina echinata Lamarck* (sample 5) by Py-CGC/MS works perfectly; while dihydroxybenzene (m/z 64, 110) and dihydroxytoluene (m/z 77, 78, 123, 124) are not observed exclusevely in brazilwood and in logwood, indenol (m/z 103, 131, 132) can be considered as a characteristic pyrolyzate of brazilin.

Summary

Two goals regarding the identification of five organic dyestuffs from the "Historical Wiesner Collection" of VUT were of great importance; on the one hand the comparison of the analytical results carried out by four laboratories using High Performance Liquid Chromatography (HPLC) for this purpose and on the other hand the status of Pyrolysis Capillary Gas Chromatography/Mass Spectrometry (Py-CGC/MS) within dyestuff analysis compared to HPLC. It was shown that laboratories working with different protocols but the same chromatographic technique (HPLC) as well as applying different methods (HPLC, Py-CGC/MS) produced very similar results concerning the identification of colouring components in dyeing materials. Consequently, Py-CGC/MS proved to be a valuable supplement to the conventional HPLC technique. It is noteworthy that analysis with Py-CGC/MS does not include any sample pre-treatment.

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