Critical Appraisal of Analytical Procedures for the Determination of Cr(VI) in Dyed Leathers by 1,5 Diphenylcarbazide Spectrophotometry After Sample Dilution or Color Removal

by

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Abstract

Colored chemical species in leather extracts may contribute to the measurement signal in spectrophotometric determination of Cr(VI), resulting in false positive Cr(VI) concentrations. In the present work the efficiency of sample dilution, or color removal by the use of RP C18 columns and columns filled with florisil, to reduce color interferences prior to spectrophotometric determinations of Cr(VI) in dyed leathers were evaluated. Limits of detections were 2.5 mg kg^{-1} Cr(VI) when samples were diluted (1:5) and 0.5 mg kg^{-1} Cr(VI) after color removal by RP C18 or florisil. In all procedures applied repeatability and reproducibility of measurements were better than ± 5 %. The accuracy of the 1,5 diphenylcarbazide spectrophotometric determination of Cr(VI) was tested by analysis of the certified reference material CRM 544, Cr(VI) in lyophilized solution. By spiking leather extracts it was experimentally proven that the use of decolorizing agents did not change Cr(VI) speciation. The experimental data also indicated that in some of the leather extracts analyzed colored species caused severe positive interference effects that could not be reduced by dilution or the use of RP C18. Florisil was found to be an efficient decolorizing agent and enabled accurate and reliable determination of Cr(VI) in dyed leathers by 1,5 diphenylcarbazide spectrophotometry.

Abstracto

Especies químicas con color en extractos provenientes del cuero, pueden contribuir a la señal siendo medida en la determinación espectrofotométrica del Cr(VI), resultando en detección de falsas positivas concentraciones de Cr(VI). En el presente trabajo la eficacia por dilución de la muestra, o remoción del color por medio de columnas de RP C18 y columnas cargadas con florisil, para reducir las interferencias por coloramiento antes de la determinación espectrofotométrica de Cr(VI) en cueros teñidos, fueron evaluadas. Los límites de detección fueron 2,5mg $Kg.^{-1}$ Cr(VI) cuando las muestras fueron diluidas (1: 5) y 0,5mg $Kg.^{-1}$ luego de descolorar por acción de RP C18 o florisil. Todos los procedimientos conllevaron a replicabilidad y reproducibilidad de mediciones superiores al ± 5%. La exactitud de la determinación del Cr(VI) espectrofotométrica por medio de 1,5 difenílcarbazida fue probada por el uso de material de referencia CRM 544, Cr(VI) en una solución liofilizada. Por medio de adiciones a los extractos del cuero se demostró experimentalmente que el uso de agentes decolorantes no cambió el contenido de la especie Cr(VI). Los datos experimentales indicaron que en algunos casos de los extractos del cuero analizados, especies coloreadas causaron efectos de severas interferencias positivas no reducibles por dilución o por el empleo de RP C18. Florisil resultó un agente decolorizante eficiente que permitió confiable y exacta detección de Cr(VI) en cueros teñidos por medio espectrofotometría con 1,5 difenílcarbazida.

Introduction

Chromium (Cr) salts are widely used as tanning agents in leather production. Among them the most frequently used is basic chromium(III) sulfate ($Cr(OH)SO_4$). In chrome tanning collagen is efficiently stabilized with Cr(III) which gives a leather of excellent quality. It also enables dyeing with a wide palette of colors, provides good water and oil resistance, as well as good cleaning properties of the leather. Chrome-tanned

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leather is soft and exhibits good durability, while the well controlled production process enables reproducible leather quality[1]. Despite several advantages of chrome tanning, problems related to safe disposal of chromium-rich tannery waste should be considered[2,3]. Leather and leather products may sometimes contain traces of hazardous Cr(VI), although only Cr(III) compounds were used in the tanning process. There are some possible sources for the occurrence of Cr(VI) in the process of leather tanning[4-7]. Cr(VI) may possibly be present as a contaminant in the tanning agent. Fat-liquoring with unsaturated fatty acids and fish oils may, after photo-ageing with UV light or thermal treatment (dry heating over 80°C), possibly lead to oxidation of Cr(III). Storage of fat-liquored leather at a relative humidity below 35% may result in Cr(VI) formation. Contamination with Cr(VI) from dyestuffs, based on Cr compounds may occur as well. Use of alkaline glues in the production of shoes may also provoke formation of Cr(VI). There are several ways to minimize the possibility of Cr(VI) occurrence in leather products[4,5]. It is recommended to use basic chromium(III) sulfate that is not contaminated with Cr(VI), to avoid the use of unsaturated natural fatty acids and fish oils, to avoid dyeing with Cr-based dyestuffs, to maintain an appropriate moisture content during leather storage, to avoid UV lighting and dry heating over 80°C, to use acidic glues in shoe production, and to use reducing agents in the final rinse bath. If the above recommendations are not entirely considered, Cr(VI) may possibly occur in leather products.

In order to prevent health risks, several legislative regulations have been issued, limiting the maximal Cr(VI) content in leather products[8-10] from 2 to 10 mg kg^{-1}, while for eco-labeled leather[11,12], the Cr(VI) concentration should not exceed 0.5 mg kg^{-1}. To comply with these legislative demands, reliable determination of Cr(VI) in leather is of extreme importance. The official method for the determination of Cr(VI) in leather is 1,5 diphenylcarbazide spectrophotometry[8-10]. Due to its liability to various interferences, e.g. the presence of colored species and colloidal particles, spectrophotometry may not always give reliable analytical results for the determination of Cr(VI) in leather extracts[13], although dilution of samples (1:5) is recommended to lower the intrinsic absorbency below a value of 0.600. The complex matrix of leather extracts (presence of collagen-chromium cross linked complexes, dyestuffs and fat liquors)[4,5,14] could also significantly influence the determination of Cr(VI) by other analytical procedures based on ion-exchange chromatography[15]. In our group, a selective analytical procedure for simultaneous determination of Cr(VI) and some negatively charged Cr(III) complexes was developed by the use of anion-exchange fast protein liquid chromatography in combination with atomic absorption spectrometry[16]. Although the procedure was successfully applied to various complex sample matrices[16-19], it was not always applicable to leather extracts due to partial overlapping of the chromatographic peaks of Cr(III) complexes with Cr(VI). Jeevan et al.[20] proposed a method that combines cleaning of the leather extract by a dialysis procedure and ion chromatography with post column derivation and spectrophotometric determination of Cr(VI). For dye removal Jambunathan and Dasgupta[21] suggested flow injection analysis based on an ion-exchange procedure followed by chlorine bleaching of the leather extract before spectrophotometric analysis. Despite the possibility of Cr(III) oxidation in the leather extract by $NaClO_4$, the authors reported that the applied procedure gave reliable results for the majority of leather extracts analyzed. Since spectrophotometry is a simple technique and may be easily adopted in industrial laboratories[22], the efforts of many researchers were oriented to overcoming interferences in determination of Cr(VI). For this purpose the possibilities of color removal were investigated. The use of a reversed phase C-18 (RP C18) columns to remove the dyes from leather extracts prior to spectrophotometric determination of Cr(VI) was suggested[10,23-25] and this procedure was proposed as an official method IUC-1810, [25,26]. Although it was widely applied[27,28], method IUC-18 was not always efficient in the removal of colored species from leather extracts[21]. Therefore, other agents were also tested. Charcoal was found to be a powerful decolorizing support, but also retained Cr(VI). Ganeshjeevan et al.[29] proposed the use of florisil as a decolorizing agent. Efficient color removal was obtained in dyed leather extracts without affecting Cr(VI) speciation. Since the IUC-18 procedure was proposed as an official method for determination of Cr(VI) in dyed leather extracts[10,25,26], the aim of our work was to compare the efficiency of RP C18 (commercially available columns) and florisil (home made columns) in color removal and the efficiency of sample dilution (1:5) in reducing colored interferences prior to determination of Cr(VI) by spectrophotometry. For this purpose twelve different dyed leather extracts were analyzed. On the basis of the efficiency of color removal and the study of the impact of decolorizing agents on Cr(VI) speciation, the accuracies and reliabilities of procedures for the determination of Cr(VI) in dyed leathers by the 1,5 diphenylcarbazide spectrophotometry were estimated.

EXPERIMENTAL

Instrumentation

A HACH DR/2010 (Lovelend, CO, USA) Portable Datalogging Spectrophotometer adjusted to a wavelength of 540 nm, using 1 cm cuvettes, was applied for the determination of Cr(VI) in leather extracts by the 1,5 diphenylcarbazide spectrophotometric method.

A Perkin Elmer (Concord, ON, Canada) Lambda 18 UV/VIS Spectrometer was used to scan the intrinsic absorbance of leather extracts before and after color removal by RP C18 or florisil in a wavelength range from 300 to 700 nm.

A WTW (Weilheim, Germany) 330 pH meter was employed to determine the pH of leather extracts.

A Vibramax 40 mechanical shaker (Tehtnica, Zelezniki, Slovenia) was used for shaking of samples.

An ISMATEC REGLO 100 (Glattbrugg, Switzerland) peristaltic pump was applied to pass the samples through the RP C18 columns and columns filed with florisil.

TABLE I
Comparison of Intrinsic Absorbance of Leather Extracts at 540 nm to Those of Diluted Leather Extracts (1:5) and of Leather Extracts After Color Removal by RP C18 or Florisil

Sample No.	Color of leather sample	Intrinsic absorbance of leather extract	Intrinsic absorbance of diluted leather extract (1:5)	Intrinsic absorbance of leather extract after color removal by RP C18	Intrinsic absorbance of leather extract after color removal by florisil
5	White	0.434±0.001	0.070±0.001	0.275±0.001	0.008±0.001
10	Beige	0.122±0.001	0.023±0.001	0.108±0.001	0.017±0.001
11	Brown	0.116±0.001	0.026±0.001	0.029±0.001	0.026±0.001
12	Red-violet	0.622±0.001	0.128±0.001	0.076±0.001	0.008±0.001
13	Red	1.092±0.003	0.243±0.001	0.696±0.001	0.067±0.001
14	Light green	0.172±0.001	0.038±0.001	0.131±0.001	0.022±0.001
15	Dark violet	3.949±0.005	1.405±0.003	2.637±0.005	0.114±0.001
16	Violet	0.057±0.001	0.012±0.001	0.019±0.001	0.012±0.001
17	Dark brown	0.483±0.001	0.093±0.001	0.106±0.001	0.021±0.001
18	Black	1.609±0.003	0.341±0.001	0.679±0.001	0.152±0.001
19	Sky blue	0.012±0.001	0.004±0.001	0.006±0.001	0.006±0.001
20	Light brown	0.631±0.001	0.140±0.001	0.543±0.001	0.199±0.001

± represent standard deviation

Reagents

Mili-Q water (Direct-Q 5 Ultrapure water system, Millipore Watertown, MA, USA) was used for preparation of samples and standard solutions. All chemicals were of analytical reagent grade.

A stock standard Cr(VI) solution (K_2CrO_4 in water, 1.000 ± 0.002 g L^{-1} of CrO_4^{2-}) was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Working Cr(VI) standards were prepared daily by dilution of the stock standard solution with water.

Dipotassium hydrogenphosphate ($K_2HPO_4 \cdot 3\ H_2O$, 0.1 mol L^{-1}) buffer solution (pH 8.0) was prepared by dissolving 22.8 g of the reagent in 1 L of water and was used as extracting solution and to adjust the pH of samples in the range from 7.5 to 8.0. The reagent was purchased from Riedel-de Haen (Hannover, Germany).

In spectrophotometric determinations Merck (Darmstadt, Germany) reagents were used. The phosphoric acid solution was prepared by dilution of 70 mL of H_3PO_4 (ρ = 1.71 kg L^{-1}) to 100 mL with water. 1,5 diphenylcarbazide ($C_{13}H_{14}N_4O$) solution was made by dissolving 1.000 g of 1,5 diphenylcarbazide in 100 mL of acetone ($CH_3(CO)CH_3$) and acidified with one drop of glacial acetic acid (CH_3COOH).

Florisil (84.0% SiO2, 15.5% MgO, 0.5% Na_2SO_4) 60/100 mesh, Supelco (Bellefonte, PA, USA) and Mega Bond Elut RP C18 columns (40°m, 1g and 5g) from Varian (Harbor City, CA, USA) were used to remove the dyes from leather extracts prior to spectrophotometric determination of Cr(VI).

Glass wool (untreated) was purchased from Supelco (Bellefonte, PA, USA).

Sartorius (Goetingen, Germany) 0.45 µm cellulose nitrate membrane filters of 25 mm diameter were used in the filtration procedure.

Sample preparation

Leather samples were cut into (1mm) strips. 2.000 ± 0.001 g of sample was shaken with 100 ml. of 0.1 mol L^{-1} K_2HPO_4 for 3 hours on a mechanical shaker at 300 rpm. The extracts were filtered through a 0.45 µm membrane filter and the pH of the samples was then measured. The pH of all leather extracts investigated ranged between 7.8 ± 0.1 and 7.9 ± 0.1. Aliquots of sample extracts were used to scan the intrinsic absorbance before and after color removal by RP C18 and florisil and for the spectrophotometric determination of Cr(VI).

Analytical procedures

Prior to spectrophotometric determination samples were either diluted (1:5) or RP C18 or florisil decolorizing agents were applied to remove the color from the leather extracts.

Recommended procedure for color removal by RP C18:

An RP C18 column (1 g) was connected to a peristaltic pump at a flow rate of 1 ml. min^{-1} and the first 10 mL were discarded. The next 20 ml. were used for the spectrophotometric determination of Cr(VI). Each column was used for only one application.

TABLE II
Recoveries of Cr(VI) in Spiked Leather Extracts After Color Removal by RP C18. Cr(VI) was Determined by the 1,5 diphenylcarbazide Spectrophotometry

Sample No.	Concentration of Cr(VI) (ng mL^{-1})	Cr(VI) added (ng mL^{-1})	Cr(VI) found (ng mL^{-1})	Recovery
5	24.5 ± 1	250 ± 5	286 ± 5	104 ± 3
10	< 10	250 ± 5	223 ± 7	89 ± 3
11	< 10	250 ± 5	238 ± 7	95 ± 3
12	15.5 ± 0.5	250 ± 5	264 ± 8	100 ± 3
13	16.5 ± 0.5	250 ± 5	248 ± 7	93 ± 3
14	< 10	250 ± 5	233 ± 7	93 ± 3
15	132 ± 4	250 ± 5	336 ± 10	88 ± 3
16	< 10	250 ± 5	238 ± 7	95 ± 3
17	12.0 ± 0.6	250 ± 5	274 ± 8	105 ± 3
18	< 10	250 ± 5	220 ± 5	88 ± 3
19	413 ± 10	200 ± 4	605 ± 20	98 ± 3
20	48 ± 2	250 ± 5	276 ± 8	93 ± 3
			Average	**96.5 ± 8**

± represent standard deviation

Recommended procedure for color removal by florisil:
Columns were made from 15 ml. (110 mm length, i.d. 15 mm) conical polyethylene tubes. A cone was drilled to make an outlet of 3 mm i.d. Glass wool was packed up to the cone (up to 5 mm) and 5 g of florisil were loaded. The column was connected to a peristaltic pump at a flow rate of 1 ml. min^{-1}. The first 10 ml. were discarded while the next 20 ml. of leather extract were used for the spectrophotometric determination of Cr(VI). Each column was used for only one application.

Recommended 1,5 diphenylcarbazide spectrophotometric procedure:
Cr(VI) was determined by the basic DIN 53314 procedure[8]. To 10 ml. of diluted (1:5) or decolorized (RP C18 or florisil) leather extract 0.5 ml. of 1,5 diphenylcarbazide and 0.5 ml. of phosphoric acid solutions were added. The magenta color was compared with that of standard Cr(VI) solutions at 540 nm after 15 min. To compensate for the intrinsic absorbance of the diluted or decolorized leather extracts, the same analytical protocol was applied, but without addition of the 1,5-dihenylcarbazide reagent. If not stated otherwise, all the analyses were made in two parallel determinations.

RESULTS AND DISCUSSION

Analytical performance of 1,5 diphenylcarbazide spectrophotometry

Several methods are available for the analysis of Cr(VI). Among them, spectrophotometry is the most popular for routine laboratories but is often subject to interferences from colored species. For routine analysis of Cr(VI) in dyed leather samples it is important to reduce color interferences efficiently before spectrophotometric determination. Therefore, the analytical performance of spectrophotometry was evaluated in leather extracts diluted 1:5 as recommended by the DIN 53314 procedure[8], and in leather extracts after color removal by RP C18 or florisil.

Linearity:
The linearity of measurement in spectrophotometric determination was obtained over a concentration range from 50 to 500 µg L^{-1} Cr(VI) with a correlation coefficient better than 0.998.

Limit of detection:
The limit of detection (LOD) for the determination of Cr(VI) in leather samples by 1,5 diphenylcarbazide spectrophotometry calculated on a 3σ basis (a value of three times the standard deviation of the blank) in decolorized leather extracts after color removal by RP C18 and florisil was 0.5 mg kg^{-1} of Cr(VI), and in diluted (1:5) leather extracts 2.5 mg kg^{-1} of Cr(VI). These LODs fulfill the legislative requirements for the determination of water-soluble Cr(VI) in leather samples of < 3 mg kg^{-1} of Cr(VI)[8] and < 10 mg kg^{-1} of Cr(VI)[10]. When decolorizing agents are applied the LOD for Cr(VI) also fulfilled the requirements for the determination of Cr(VI) in Cr-free leather of < 0.5 mg kg^{-1} of Cr(VI)[11] and general requirements for gloves of < 2 mg kg^{-1} Cr(VI)[9].

Repeatability of measurement:
The repeatability of measurement for Cr(VI) was tested for samples No. 19 and 20 by six consecutive determinations of hexavalent Cr on diluted (1:5) or decolorized extracts by RP C18 or florisil. It was found to be the same for two samples analysed and was ± 2 %, for diluted (1:5) extracts, ± 2 % for decolorized extract by RP C18 and ± 1.5 % for decolorized extract by florisil (± values represented standard deviation).

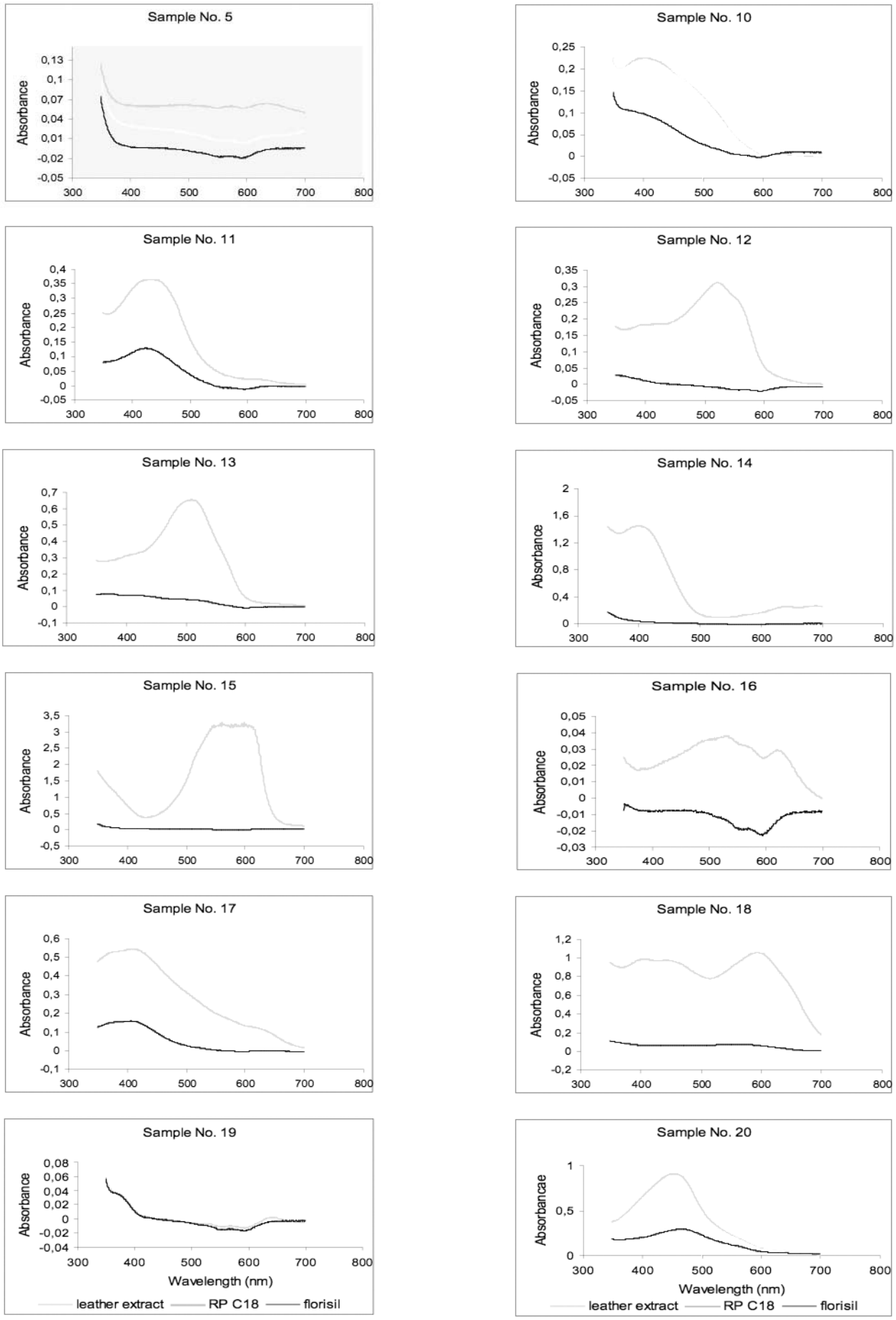

FIGURE 1 - UV scans of intrinsic absorbances of leather extracts, leather extracts after colour removal by RP C18 and leather extracts after colour removal by florisil over the wavelength range from 300 to 700 nm. The colours of leather samples are given in Table I.

TABLE III
Recoveries of Cr(VI) in Spiked Leather Extracts After Color Removal by Florisil. Cr(VI) was Determined by the 1,5 diphenylcarbazide Spectrophotometry

Sample No.	Concentration of Cr(VI) (ng mL^{-1})	Cr(VI) added (ng mL^{-1})	Cr(VI) found (ng mL^{-1})	Recovery
5	12.2 ± 0.5	250 ± 5	255 ± 7	97 ± 3
10	< 10	250 ± 5	234 ± 7	93 ± 3
11	< 10	250 ± 5	238 ± 7	95 ± 3
12	12.8 ± 0.5	250 ± 5	256 ± 8	97 ± 3
13	16.1 ± 0.5	250 ± 5	265 ± 8	100 ± 3
14	11.7 ± 0.5	250 ± 5	244 ± 8	93 ± 3
15	< 10	250 ± 5	230 ± 7	92 ± 3
16	15.5 ± 0.5	250 ± 5	256 ± 8	96 ± 3
17	11.7 ± 0.5	250 ± 5	253 ± 8	97 ± 3
18	< 10	250 ± 5	257 ± 8	103 ± 3
19	400 ±10	200 ± 4	611 ± 15	102 ± 3
20	48 ± 2	250 ± 5	310 ± 10	104 ± 3
			Average	**98 ± 4**

± represent standard deviation

Reproducibility of measurement:
The reproducibility of measurement for Cr(VI) was tested for samples No. 19 and 20 by six consecutive determinations of hexavalent Cr on two different days. Diluted (1:5) or decolorized extracts by RP C18 or florisil were examined. The reproducibility of measurement was found to be the same for two samples analysed and was ± 5 % for diluted (1:5) extracts, ± 5 % for decolorized extract by RP C18 and ± 4 % for decolorized extract by florisil (± values represented the range of data between two different days).

Accuracy check:
The accuracy of the spectrophotometric determination of Cr(VI) was tested by the analysis of certified reference material CRM 544, Cr(VI) in lyophilised solution. The result expressed as average of six consecutive determinations of hexavalent Cr ± standard deviation indicated good agreement between the value determined (23.9 ± 1.0 ng mL^{-1} Cr(VI)) and the certified value (22.8 ± 1.0 ng mL^{-1} Cr(VI)).

The efficiency of color removal by RP C18 and florisil
In order to evaluate the efficiency of color removal by RP C18 and florisil, twelve leather extracts with different leather colors were investigated. The intrinsic absorbance of leather extracts and leather extracts after color removal by RP C18 or florisil were scanned in the wavelength range from 300 to 700 nm. The scans are presented in Figure 1, while in Table I intrinsic absorbance of leather extracts at 540 nm, of diluted leather extracts (1:5) and of leather extracts after color removal by RP C18 or florisil are compared. The scans of Figure 1 clearly demonstrate that florisil was more effective in color removal than RP C18 and that in some samples e.g. 13 (red), 15 (dark violet) and 18 (black) poor efficiency of RP C18 was observed. It should be pointed out that the amount of RP C18 agent was 1 g as it is recommended in IUC-18 procedure[10]. It was further experimentally proven that the efficiency of color removal in leather samples was not better even when 5 g RP C18 columns were applied. These findings are in agreement with the observations of Jambunathan and Dasgupta[21] who reported that IUC-18 method based on color removal by RP C18 is not always efficient. From the data of Table I it is further evident that at the wavelength of Cr(VI) determination (540 nm) sample dilution (1:5) or decolorizing by RP C18 are not as effective as the use of florisil. In general, the latter decolorizing agent significantly reduces the intrinsic absorbance of leather extracts. This effect is particularly pronounced in the dark colored leather extracts of samples No. 12 (red-violet), 13 (red), 15 (dark violet), 17 (dark brown) and 18 (black), indicating the great efficiency of florisil in color removal.

Investigation of the impact of decolorizing agents on Cr speciation
To investigate the influence of color removal on Cr speciation, aliquots of leather extracts were spiked with Cr(VI). Spiked and non-spiked samples were passed through RP C18 or florisil columns. After the decolorizing procedure Cr(VI) in spiked and non-spiked leather extracts was determined by spectrophotometry. The recoveries, calculated as the ratio between the Cr(VI) concentration found and that added, are presented in Tables II and III (color removal by RP C18 and florisil, respectively).

It is evident that recoveries for RP C18 lie between 88 and 105 % (average 96.5 ± 8) while for florisil they are between 92 and 104 % (average 98 ± 4). These data indicate that during the decolorizing procedure Cr(VI) is neither adsorbed nor reduced

TABLE IV
Determination of Cr(VI) Concentrations* in Leather Samples by the 1,5 diphenylcarbazide Spectrophotometry. Prior to Analysis Leather Extracts Were Either Diluted 1:5 or Decolored by RP C18 or Florisil

Sample No.	Color of leather sample	Concentration of Cr(VI) (mg kg^{-1}) leather extract diluted 1:5	Concentration of Cr(VI) (mg kg^{-1}) color removal by RP C18	Concentration of Cr(VI) (mg kg^{-1}) color removal by florisil
5	White	< 2.5	1.22 ± 0.06	0.61 ± 0.02
10	Beige	< 2.5	< 0.5	< 0.5
11	Brown	< 2.5	< 0.5	< 0.5
12	Red-violet	< 2.5	0.78 ± 0.04	0.64 ± 0.02
13	Red	< 2.5	0.83 ± 0.04	0.81 ± 0.02
14	Light green	< 2.5	< 0.5	0.58 ± 0.02
15	Dark violet	21.4 ± 0.9	6.6 ± 0.3	< 0.5
16	Violet	< 2.5	< 0.5	0.78 ± 0.02
17	Dark brown	< 2.5	0.60 ± 0.04	0.58 ± 0.02
18	Black	< 2.5	< 0.5	< 0.5
19	Sky blue	19.7 ± 0.9	20.6 ± 0.08	20.0 ± 0.6
20	Light brown	1.89 ± 0.09	2.39 ± 0.09	2.39 ± 0.07

* mean of three parallel determinations ± standard deviation

by the column support. Therefore, RP C18 and florisil do not influence Cr(VI) speciation. The accuracy and reliability of Cr(VI) determination by spectrophotometry depends primarily on the efficiency of color removal from the leather extracts.

Analysis of Cr(VI) in diluted and decolorized leather extracts by 1,5 diphenylcarbazide spectrophotometry

A comparison of Cr(VI) concentrations in leather samples determined by 1,5 diphenylcarbazide spectrophotometry after dilution (1:5) of leather extracts or decolorization by RP C18 or florisil is given in Table IV. The data indicate that in the majority of samples analyzed after dilution (1:5) of leather extracts, Cr(VI) concentrations were < 2.5 mg kg^{-1}. In these samples Cr(VI) concentrations determined after the decolorizing procedures were either < 0.5 mg kg^{-1}, or ranged from 0.6 to 1.2 mg Cr(VI) kg^{-1}, and the RP C18 and florisil decolorizing procedures agreed well. An exception was sample No. 5 (white) where more efficient color removal by florisil resulted in a lower Cr(VI) concentration. Excellent agreement between all three procedures was obtained in light colored sample No. 19 that contained about 20 mg Cr(VI) kg^{-1}. This Cr(VI) concentration significantly exceeded the maximal permitted legislative values[8-10]. The worst disagreement of results was found in sample No 15. Due to its dark violet color, the intrinsic absorbance of the leather extract of 3.949 (see Table I) was not efficiently reduced by sample dilution 1:5. The resulting intrinsic absorbance after sample dilution was 1.405 and did not follow the dilution factor of 5. For that reason a false positive Cr(VI) concentration of 21.4 mg kg^{-1} was determined. Color removal by RP C18 was also not effective (intrinsic absorbance 2.637) resulting in a false positive Cr(VI) determination of 6.6 mg kg^{-1}. Efficient color removal (intrinsic absorbance 0.114) in this sample was obtained by the florisil decolorizing agent. Therefore, it may be concluded that the Cr(VI) concentration determined of < 0.5 mg kg^{-1} is accurate and reliable. Analysis of sample No. 15 also confirmed the findings of Jambunathan and Dasgupta[21] who reported that color removal by RP C18 is not always efficient, and of Milacic et al.[13] who confirmed that dilution of leather extracts may not necessarily reduce interferences of colored species for reliable determination of Cr(VI). On the basis of the present investigation it may be concluded that florisil is a more powerful decolorizing agent than RP C18 and enables accurate and reliable determination of Cr(VI) by spectrophotometry. Our findings are in agreement with the recommendations of Ganeshjeevan et al.[29] who suggested the use of florisil as a decolorizing agent prior to spectrophotometric determination of Cr(VI) in dyed leathers.

CONCLUSIONS

In the present study spectrophotometric determination of Cr(VI) after sample dilution (1:5) or color removal by RP C18 or florisil were critically evaluated. It was experimentally proven that florisil was more effective in color removal than RP C18. On the basis of the recovery tests of spiked leather extracts it was found that none of the decolorizing agents influenced Cr(VI) speciation. Analysis of Cr(VI) in dyed leathers gave good agreement of results in samples where both decolorizing procedures were efficient and where sample dilution (1:5) appreciably reduced the intrinsic absorbance of the leather extract. However, in some of colored leather extracts analyzed dilution of samples (1:5) and RP C18 were not effective at all,

resulting in false positive determinations of Cr(VI), although the latter procedure is recommended to become an official method for the determination of Cr(VI) in dyed leathers[10,25,26]. Florisil enabled efficient color removal in all samples analyzed and accurate and reliable determination of Cr(VI). In addition, sensitive determination of Cr(VI) in dyed leathers is achieved after color removal by florisil (LOD 0.5 mg kg^{-1} Cr(VI)), thus also fulfilling the most restrictive legislative requirements for the determination of hexavalent Cr in chromium-free leathers. Therefore, the use of florisil as a decolorizing agent is recommended prior to spectrophotometric determination of Cr(VI) in dyed leathers.

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