Ion Chromatography with Post Column Derivatization for the Determination of Hexavalent Chromium in Dyed Leather. Influence of the Preparation Method and of the Sampling Location

by

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Abstract

Since 2015, a European restriction limits the hexavalent chromium content to not more than 3 mg/kg in leather products (UE-301-2014, 25 March 2014, §47, annex XVII of REACh). Owing to spectrophotometric interferences encountered with the established European standard EN ISO 17075:2007 when colored leathers have to be analyzed, this article proposes the analysis of hexavalent chromium using ion chromatography with post-column derivatization and spectrophotometric detection. In order to avoid any pre-treatment of the crude extract an on-line solid phase extraction step is introduced by inserting a high capacity reversed phase guard column. The efficiency of the on-line purification is demonstrated (quantitative removal of the dye present in the extract) and allows the direct analysis of crude extracts in less than 6 minutes. The overall chromatographic method is validated following procedures described by the French standard NF T90-210. The measurement error (bias) was determined at 7.2% at the presupposed limit of quantification 5 µg/L (corresponding to a hexavalent chromium content of 0.25 mg/kg in leather) and at the medium concentration level and at 4.0% for the high-level concentration, with relative standard deviations of 5.1%, 2.9% and 2.4% respectively. The recovery yields measured with spiked leathers range from 92 to 96% depending on the hexavalent content. The reliability of the method is also demonstrated through two inter-laboratories test involving several European laboratories. The crucial influence of the sample preparation is also assessed in this work. It is demonstrated that the hexavalent chromium content of leathers is systematically underestimated when the extraction is applied to leathers cut in small pieces and not ground. In parallel, additional tests on leather sampling location show that the shoulder zone is not representative of the rest of the skin in terms of hexavalent chromium content since it has a higher hexavalent chromium content than all other sampling zones.

Introduction

In the leather industry, more than 80% of the leathers are tanned with chromium compounds because this manufacturing process results in excellent physio-mechanical properties for leather. Chromium is a transition metal existing in several oxidation states, trivalent and hexavalent chromium being the most common forms. The chemical properties of chromium depend on its oxidation state. Whereas trivalent chromium is a harmless chemical form for the health, hexavalent chromium is much more toxic. Although only a trivalent chromium salt solution (at a high concentration of about 7%) is commonly used in the tanning process, a small fraction of it can be oxidized into the hexavalent form and therefore be detected in the finished leather products. The fraction of trivalent chromium involuntary oxidized into hexavalent chromium may depend on production as well as on the storage conditions of leather. The hexavalent chromium is a known allergen and even traces of this compound may cause sensitization and irritation when exposed to human skin.[1-3] Consequently, the European Union has acted a directive to limit the presence of hexavalent chromium at 3 mg/kg in the leather produc.[4,5]

Three test methods (International Union of Chemistry IUC 18, the Deutsches Institut für Normung DIN 53314 and the European Norm EN 420) based on the same principle for the analysis of hexavalent chromium were published in the nineties. The hexavalent chromium is extracted from ground leather in phosphate buffer degassed at pH 8. After three hours of extraction, the extracted hexavalent chromium is complexed with diphenylcarbazide in acidic solution which produces a red-purple colored complex. The concentration of hexavalent chromium is quantified by its absorbance measurement at 540 nm.[5,6] The lack of robustness of this method and its inability to quantify a concentration level as low as 3 mg/kg has been quickly identified for the colored leathers.[6,7] Several years later, the International

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Organization for Standardization ISO and Comité Européen de Normalisation CEN published jointly the reference standard EN ISO 17075:2007. On this current standard, the principle was similar but a purification step (extraction of the dye by a solid phase extraction step on a reversed phase cartridge) was added to eliminate the interfering dyes. These modifications allowed reaching the 3 mg/kg quantification limit.[8]

Recently, several industry branches have proposed ion chromatography with spectrophotometric detection for a more reliable analysis of hexavalent chromium in water,[9] fertilizers,[10] cement,[11] wastes and soils[12] and cosmetics products.[13] Our article will explore the potential of this chromatographic approach and its optimization and validation for the analysis of leather. Although the anion-exchange chromatographic step allows separating the hexavalent chromium from co-extracted species, sample off-line pre-treatments were always reported as mandatory in previous studies.[14,15] In the first study,[14] the authors demonstrated that the high loading of dyes in the leather extracts had a severe detrimental effect on sensitivity and peak shapes and poisoned the ion-exchange stationary phase irreversibly. They proposed an off-line dialysis step to remove interfering compounds (essentially dyes with a high molecular weight). In the second study,[15] an off-line solid phase extraction sample pre-treatment was proposed to eliminate chemical and physical interferences.[15] Such off-line pretreatments are time consuming, require costly sample handling and may be sources of error. Therefore, the introduction of an on-line purification step is proposed in this study by inserting a high capacity reversed-phase guard column (Thermo Scientific™ Dionex™ IonPac NG1) before the analytical column. After the separation step, the detection of the hexavalent chromium will be implemented after a post-column reaction step. Thus, the chromatographic effluent will be submitted to a post-column derivatization step with diphenylcarbazide in acidic solution to produce the red-purple colored complex detected by spectrophotometry. The combination of an on-line reversed-phase purification step with an anion-exchange chromatographic step and a post-column derivatization should ensure a great specificity, sensitivity and accuracy for the developed method.

First, the capacity of the reversed-phase guard column to remove the dye from the colored leather extracts will be evaluated. Then, the full validation of the analytical method (linearity, limit of quantification, accuracy and recovery) will be realized according to the French standard NF T90-210.[16] To complete the statistical evaluation of this chromatographic method, two European inter-laboratories test involving several laboratories will be realized to evaluate its reliability. In addition, a final study will demonstrate the critical influence of the sampling step (influence of the sampling location on the skin) and of the preparation step (cut or ground leather) on the hexavalent chromium content measured.

Experimental Procedures

Chemicals

All reagents used during analysis are of analytical grade purity: Potassium phosphate dibasic trihydrate K_2HPO_4, $3H_2O$ at 99% (Acros Organics, Geel, Belgium), ammonium sulfate at 99% (Fisher Scientific, Loughborough, UK), 1,5 diphenylcarbazide (CAS 140-22-7) at 98% (Fisher Scientific, Loughborough, UK), orthophosphoric acid at 70% (Fisher Scientific, Loughborough, UK), sulfuric acid at 95% (VWR, Fontenay-sous-Bois, France), methanol (VWR, Fontenay-sous-Bois, France), ammoniac at 28% (VWR, Fontenay-sous-Bois, France), standard solution of hexavalent chromium at 1000 mg/L in water (Analytika, Prague, Czech Republic), Standard control solution of hexavalent chromium at 1000 mg/L in water (TechLab, Metz, France).

Sample Preparation

The sample preparation steps of the leather are identical to the current standard EN ISO 17075:2007. As a reminder, 2 grams of leather (cut or ground as explained in the text) are suspended in 100 mL of extraction buffer for sample extraction. Dipotassium hydrogen phosphate trihydrate is used for the extraction buffer by dissolving 22.8 g in 900 milliliters of deionized water. The buffer is adjusted at pH 8.0 ± 0.2 with orthophosphoric acid and made up to 1 liter. Before extraction, the buffer is degassed with an inert gas (argon or nitrogen) to prevent the oxidation of trivalent chromium into hexavalent chromium by oxygen. After 3 hours of stirring, the extract is immediately filtered using a 0.45 µm nylon filter. An aliquot of the filtered extract is analyzed using ion-exchange chromatography with post column derivatization.

Ion Chromatography

Equipment

The ion-exchange chromatography analysis was performed with Thermo Scientific™ Dionex™ ICS 5000+ Ion Chromatographic system (Massachusetts, USA) configured with two dual pumps (for mobile phase and for post column reagent) and a Diode Array Detector (DAD) for UV-VIS detection (PEEK, cell volume 11 µL; light path length 10 mm, wavelength 540 nm). The hexavalent chromium was analyzed using a guard column (Thermo Scientific™ Dionex™ IonPac NG1, 35 mm* 4 mm i.d.) and an analytical column packed with a quaternary ammonium anion exchange stationary phase (Thermo Scientific™ Dionex™ IonPac AS7, 250 mm*4 mm i.d.) at a temperature-controlled of 30°C and using an injection loop of 100 µL. The post-column reaction between hexavalent chromium and diphenylcarbazide is realized in a reaction coil (Thermo Scientific™ Dionex™, 750 µL).

Preparation of the Mobile Phase

The mobile phase is a 250 mM ammonium sulfate and 100 mM ammonium hydroxide solution, prepared using 33.0 g of ammonium sulfate and 8 mL of 28% ammonium hydroxide solution in 1 L of deionized water. The elution is realized in the isocratic flow mode at 1 $mL.min^{-1}$.

Preparation of Post-column Reagent

The post-column reagent is a 2 mM diphenylcarbazide and 1 N sulfuric acid solution containing 10% of methanol prepared as follows. In a 1000 mL volumetric flask, 28 mL of 98% sulfuric acid are dissolved in 500 mL of deionized water. 0.50 g of 1, 5-diphenylcarbazide are dissolved in 100 mL of methanol. When the acidic solution is cooled, the diphenylcarbazide solution is added into the 1 L volumetric flask and diluted to 1 L with deionized water. The flow rate of the post-column reagent is set at 0.33 $mL.min^{-1}$.

Results and Discussion

On-line Reversed Phase Solid Phase Extraction for Dye Removing

Dyed leathers present particular challenges for the determination of hexavalent chromium when spectrophotometric detection is used. A part of the dyes present in the leather may be co-extrated with hexavalent chromium during the 3 hours extraction step. The detection (at 540 nm) of the red-purple complex formed between hexavalent chromium and diphenylcarbazide during the post-column reaction step may be interfered by the dyes that absorb in this wavelength range i.e. by red dyes. To reduce the detrimental impact of red dyes on the analysis, the current standard EN ISO 17075:2007 recommends the use of purification cartridge (reversed-phase cartridge) before the direct spectrophotometric determination. Although the introduction of a chromatographic separation step should allow separating the hexavalent chromium from the other interfering compounds present in the extract, an on-line solid phase extraction purification step was added to preserve the analytical column and suppress all pre-treatment requirements. Therefore, an appropriate guard column was inserted before the analytical columns to protect it from hydrophobic compounds and to remove anionic/hydrophobic dyes co-extracted in the samples.

This guard column (IonPac NG1 guard column), packed with polystyrene divinylbenzene particles (PS-DVB), operates according to a reversed phase mechanism. This polymeric support allows using 100% aqueous and highly basic mobile phases, an advantage over silica chromatographic supports that undergo dissolution in such media. In order to demonstrate the efficiency of the IonPac NG1 guard column to remove the dyes present in the extraction buffer, an acidic dye (anionic dye in the extraction conditions) was selected among the most intensely red-colored extracts obtained in the usual conditions of sample preparation discussed earlier. The dye studied is composed of Acid Red 97 (CAS 10169-02-5) and Acid Red 131 (CAS 70210-37-6) absorbing respectively at the wavelengths of 530 nm and 550 nm. The extract of a red-colored leather (free of hexavalent chromium) was injected with and without the guard column (100 µL for each injection) and directly detected at 540 nm. As illustrated Figure I (left), the dye saturates the spectrophotometric detector at 5000 mAU when the colored extract is injected without the guard column. After the introduction of the guard column, the situation is quite different. The right picture in Figure 1 shows the signals recorded for the first and the 150th injection without any regeneration of the guard column in-between these 150 several successive injections. After 150 repeated injections of the same colored extract, the low signal at 7 mAU demonstrates that the dye is always almost quantitatively retained on the guard column. The same experiment (not presented in this paper) was carried out using a metal complex dye Brown HH 469 (CAS 400-320-4), highlighting still the high capacity of the guard column. In absence of the guard column, the anionic/hydrophobic dyes are fully retained by the polymeric and cationic support of the analytical column (by anion-exchange mechanism and by hydrophobic effect) and poison it. These results highlight the efficiency and great capacity of the guard column. Indeed, although a frequent cleaning (with a mixture of methanol/ water (70/30; v/v) at flow rate of 1 $mL.min^{-1}$ for approximately 30 minutes) of the guard column Ion Pac NG1 is recommended by the supplier, 150 injections can be done without any regeneration. Given a total analysis time of about 10 minutes a daily regeneration is enough even in the case of solutions highly charged with dyes.

Validation of the Analytical Method

The overall method was validated according to the French standard NF T90-210 including the linearity, the accuracy and the recovery of the method.

Linearity

Five-point calibration curves were prepared in the extraction buffer using a standard solution of hexavalent chromium at

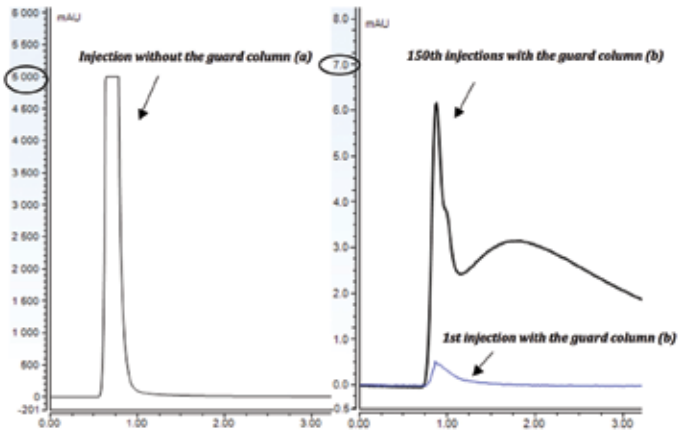

Figure 1. Efficiency of the guard column.
Direct injection of an extract containing a red dye (a) without any guard column, (b) with the guard column IonPac NG (135mm x 4mm). Mobile phase of 250 mM ammonium sulfate and 100 mM ammonium hydroxide. Constant flow of mobile phase 1 mL/min. Injection volume 100 µL. Oven 30°C. Detection at 540 nm.

1000 mg/L. Hexavalent chromium concentrations ranged from 5 to 1000 µg/L, corresponding to 0.25 to 50 mg/kg of hexavalent chromium content in the total weight of the leather. The model linearity was validated by maximum accepted limits on five inter-day calibrations. In this study, the maximum accepted deviation was fixed at ±20% for the two lowest points of the concentration range (5 and 10 µg/L) and ±10% for the other points (50, 250, and 1000 µg/L). As illustrated in Figure 2, the bias between the reference concentrations and the recalculated concentrations of hexavalent chromium is acceptable for all the concentration levels studied. For information, the linear calibration curves were generated with coefficients of determination greater than R^2=0,999. An example of chromatogram at 5 µg/L of hexavalent chromium (the presupposed LOQ) is presented in Figure 3. This LOQ (5 µg/L) for hexavalent chromium corresponds to a LOQ of 0.25 mg/kg of hexavalent chromium when considering that 2 grams of leather are extracted with 100 mL of extracting solution. Thus, this method fulfils the requirements since the validated detection limit is 20 times lower than the maximum acceptable content (3 mg/kg).

Method Accuracy

The accuracy was verified spiking a leather sample (selected in a lot of chrome-tanned leathers free of hexavalent chromium) using the standard solution of hexavalent chromium at 1000 mg/L. The accuracy was verified at the limit of quantification (5 µg/L), the medium and high concentrations (50 and 1000 µg/L). The sample preparation procedure described earlier was followed for each sample. Two determinations were performed on five different days for the three concentration levels. The accuracy test can be validated with maximum accepted deviations of ±60% at 5 µg/L (the limit of quantification), of ±40% at 50 µg/L and of ±20% at 1000 µg/L. The performance of the method was evaluated according to the relative standard deviation (%) and the measurement error (bias %) between the reference concentrations of hexavalent chromium (known spiked level) and the measured concentrations in samples. The measurement error (bias) was determined at 7.2% at the limit of quantification and medium level and at 4.0% for the high level, with relative standard deviations of 5.1%, 2.9% and 2.4% respectively for the three concentration levels. Thus, the measured values did not exceed the maximum accepted deviations for each concentration level.

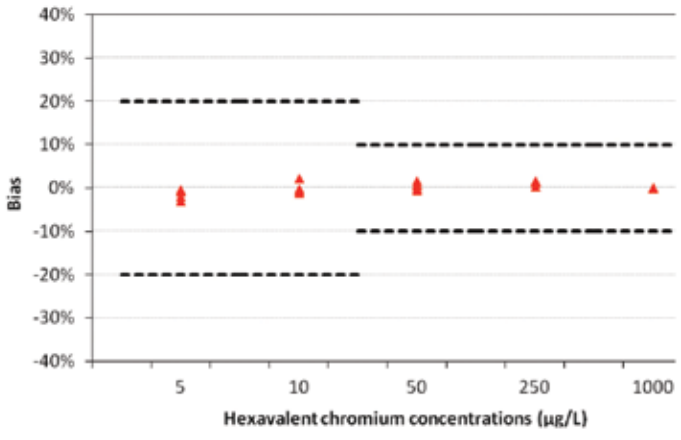

Figure 2. Observed bias of five point calibration curves.
Determination of bias between the reference concentrations and the recalculated concentrations of hexavalent chromium. 5 points calibration curves prepared in the extraction buffer x 5 days. Dotted lines represent the maximum accepted deviations.

Method Recovery

Finally, the extraction recovery yields were evaluated at each concentration level and must be between 60% and 120%. The method recoveries were determined using the same set of experiments than in the accuracy evaluation. The recovery yields range from 92 to 96%.

Evaluation of the Reliability of IC Through an Inter-laboratory Test

Inter-laboratory Test 2014

An inter-laboratory test was organized between several European laboratories to evaluate the relevance of the different test methods for the determination of hexavalent chromium. The test compares the current colorimetric method EN ISO 17075:2007 (called C) and the method using a chromatographic step (called IC –PCD for ion chromatography with post column detection and IC-DD for ion chromatography with direct detection).

A sample of bovine leather (not colored) was analyzed in duplicate by 15 European laboratories using their own method of analysis. In order to homogenize the sample, the leather was cut

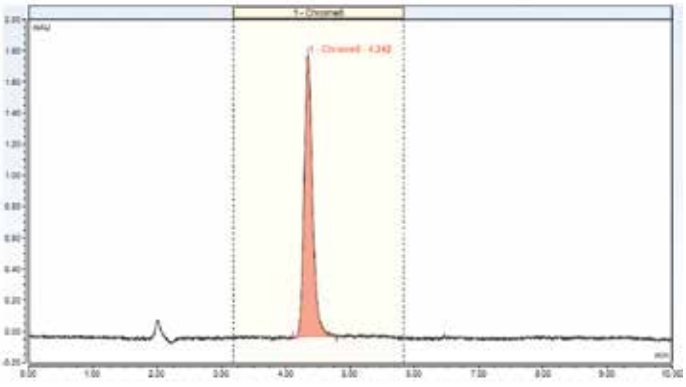

Figure 3. Chromatogram at 5 µg/L of hexavalent chromium concentration.
Column IonPac AS7 (250mm x 4mm) and guard column IonPac NG (135mm x 4mm). Mobile phase of 250 mM ammonium sulfate and 100 mM ammonium hydroxide. Constant flow of mobile phase 1 mL/min. Post-column reagent of 2 mM diphenylcarbazide and 1 N sulfuric acid solution containing 10% of methanol. Constant flow of post-column reagent 0.33 mL/min. Injection volume 100 µL. Oven 30°C. Detection at 540 nm.

in pieces of 3-5 millimeters that were mixed before to be dispatched (approximately 5 grams of leather for each participating laboratory).

The individual performance of each laboratory was evaluated on the accuracy of its results using the Z-score. The Z-score was calculated using the measured concentration in the sample (average of two replicates noted x), the mean concentration of all laboratories (μ) and its standard deviation (σ) as following:

$$Z_{score} = \frac{x - \mu}{\sigma}$$

The Z-score is considered satisfactory when its absolute value is less than or equal to 2. The results for all laboratories are summarized in Table I. The mean value between all laboratories is 3.4 mg/kg and the standard deviation is 1.1 mg/kg of hexavalent chromium. The Z-scores are satisfactory for all participating laboratories including for the laboratory number 15 represented in this paper. The inter-laboratory trial show that the results are equivalent between all laboratories whatever the method used.

Inter-laboratory Test 2016

In 2016, a second inter-laboratory test was realized between the European laboratories using exclusively the chromatography methods. As in previous tests, 2 grams of homogeneous leather cut in pieces of 3-5 millimeters of bovine leather (not colored) was analyzed in duplicate by seven laboratories. The results are presented in Table II. The mean concentration is 6.6 mg/kg with a standard deviation of 1.6 mg/kg of hexavalent chromium. The values of Z-score demonstrate the good performance of the test method for the seven laboratories (Z-score of 0.1 for our laboratory called C). This study proves the reliability of the chromatographic method for the determination of hexavalent chromium in leathers. The choice of the detection method, with or without post-column derivatization, depends on the required sensitivity, the direct detection of chromates at 372 nm being less sensitive than the detection after post-column derivation by diphenylcarbazide.[11]

Influence of the Sample Preparation for the Analysis

In the EN ISO 17075:2007 method, the leather samples are prepared according to the standard ISO 4044:2008 that recommends the analysis on ground leather. However, for leathers containing glue or for leathers with a smaller quantity available (e.g. the shoes leather) the grinding step is problematic; to obtain 2 grams of ground leather it is necessary to introduce two or three-fold the quantity of raw leather. In that case, the leather is preferentially cut for the analysis of hexavalent chromium. In order to highlight the effect of the sample preparation, the next study compares the influence of these two methods of sample preparation referred as "grinding" and "cutting." 24 extracts were prepared with twelve leathers randomly selected in a lot of chrome-tanned leathers. For each leather, 2 grams of ground or cut leather (pieces from 3-5 mm) were extracted in parallel. The results summarized in Figure 4 show that the amount of hexavalent chromium measured in the ground sample is significantly higher (by a factor of 1.2 to 1.8) than the one obtained with the finely cut sample on the same leather. The higher extraction yield of hexavalent chromium may be attributed to the larger contact surface of the ground sample. To verify the influence of the surface area, contact on the

Table I
Inter-laboratories test between 15 European laboratories in 2014. Results of hexavalent chromium (mg/kg)

Laboratories	Analysis method	Measured concentration	Z_{score}
1	C	3.9	0.5
2	IC DD	2.1	-1.2
3	IC DPC	2.6	-0.8
4	C	3.6	0.2
5	C	3.7	0.3
6	C	3.4	0.1
7	C	4.5	1.0
8	C	3.9	0.4
9	C	3.9	0.4
10	C	4.8	1.3
11	C	5.3	1.7
12	C	1.8	-1.5
13	IC DD	1.6	-1.7
14	C	3.2	-0.2
15	IC DPC	4.0	0.6

Mean concentration = 3.4 mg/kg ; Standard deviation = 1.1 mg/kg of hexavalent chromium.
C: Colorimetric method (according EN ISO 17075)
IC DD: Ion Chromatography with Direct Detection
IC PCD: Ion Chromatography with Post Column Derivation.
Z_{score} is calculated using the measured concentration in the sample (average of two determinations), the mean concentration of all laboratories and its standard deviation.

extraction yield and evaluate the necessity to grind or not the sample, complementary experiments were implemented. A leather sample (called leather 13) was divided into four samples that were either ground or cut in pieces of various dimensions ranging from 2-3 mm, 3-5 mm to 6-7 mm side length. The hexavalent chromium contents of these samples were determined and are summarized in Table III. They confirm that the hexavalent chromium content measured is higher when decreasing the size of the cut pieces and tends towards the value obtained for the ground sample. In other words, the hexavalent chromium content may be underestimated on the cut leathers. The study highlight that the piece size plays a critical role in the extraction recovery of hexavalent chromium and that this step of sample preparation must be controlled.

Influence of the Sampling Location on an Entire Skin

The standard ISO 2418:2002 specifies the location of the sampling zone within a skin depending on the piece of the skin available: the whole hide, the shoulder, the belly, the bend…. This standard is applicable to all kinds of leather derived from mammals, irrespective of the tanning process used. The aim of this part was to study the distribution of hexavalent chromium in the whole skin.

Table II
Inter-laboratories test between 7 European laboratories in 2016. Results of hexavalent chromium (mg/kg)

Laboratories	Analysis method	Measured concentration	Z_{score}
A	IC DPC	8.5	1.0
B	IC DPC	9.1	1.4
C	IC DPC	6.7	0.1
D	IC	5.2	-0.8
E	IC DD	5.3	-0.8
F	IC	6.0	-0.4
G	IC	5.6	-0.6

Mean concentration = 6.6 mg/kg ; Standard deviation = 1.6 mg/kg of hexavalent chromium.
IC: Ion Chromatography (Detection mode not known)
IC DD: Ion Chromatography with Direct Detection
IC PCD: Ion Chromatography with Post Column Derivation.
Z_{score} is calculated using the measured concentration in the sample (average of two determinations), the mean concentration of all laboratories and its standard deviation.

13 small pieces of leather were sampled (as illustrated Figure 5) in the same bovine leather, randomly selected in a lot of chrome-tanned leathers. The sampling zone 9 (in the bend) corresponds to the location area recommended in the standard 2418:2002. This sampling zone did not contain any hexavalent chromium compound (less than the LOD i.e. less than 0.1 mg/kg). In the rest of the skin, the concentration of hexavalent chromium was also below 0.3 mg/kg except for the sampling zone 1 that released a larger amount of hexavalent chromium of about 3.9 mg/kg. This preliminary study shows that the hexavalent chromium content in the sampling 9 is representative of the whole skin except for a more sensitive zone, the shoulder zone, which seems to be prone to the formation of hexavalent chromium.

In order to confirm or not this trend, this study was completed by the analysis of other 80 bovine leathers from a French footwear manufacturer. In each leather, a sampling was realized in the bend (sampling 9) and in the shoulder (sampling 1) of the skin. 72 leathers (90% of the leathers) conform to the legislation with a of hexavalent chromium content less than 3 mg/kg, whatever the sampling zone in the skin (results not shown). As illustrated in Figure 6, among the eight remaining leathers (called Leather 1 to 8) that contained more than 3 mg/kg of hexavalent chromium in the shoulder, only 4 were above the limit of 3 mg/kg in the rest of the skin (expect for the leather 4).

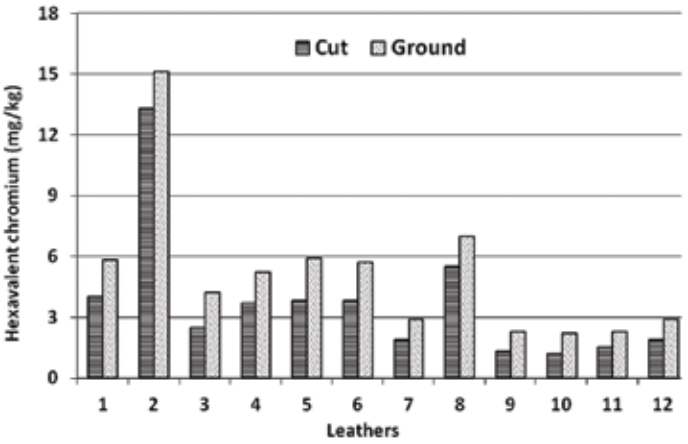

Figure 4. Influence of the leather preparation mode on the hexavalent chromium content measured.

Table III
Influence of the piece size on the hexavalent chromium content measured. Results of hexavalent chromium (mg/kg)

	Ground	2-3 mm	3-5 mm	6-7 mm
Leather 13	14.5	11.7	7.3	7.2

In other words, 10% of leathers were tested positive in the shoulder zone against only 5% in the bend zone. These tests of sampling location confirm that the shoulder area seems to be more sensitive and presents an increased risk of formation of hexavalent chromium. Several explanations may account for this difference. The quantity of fat liquor is higher in the animal shoulder and may promote the oxidation of trivalent chromium in hexavalent chromium.[17,18] However, the results (not shown) of the determination of the fat liquor in these leathers (in accordance with ISO 4048:2008 determination of the substances in leather which are soluble in dichloromethane) did not support this first assumption (no correlation could be evidenced). The fibers structure of collagen can also be suspected. Indeed, the fibers located in the extremity of the skin are usually less dense and less tight than in the skin center. This structure difference between the shoulders area and bend area in a same skin is visually illustrated in the Figure 7. Therefore, two consequences can be mentioned. First, the penetration of an oxidizing agent may be easier through the fibers less tight and may influence the oxidation of trivalent chromium in the leather. The second hypothesis concerns the extraction step where the extraction solution will wet more completely the leather and can be increased the extracted quantity of hexavalent chromium.

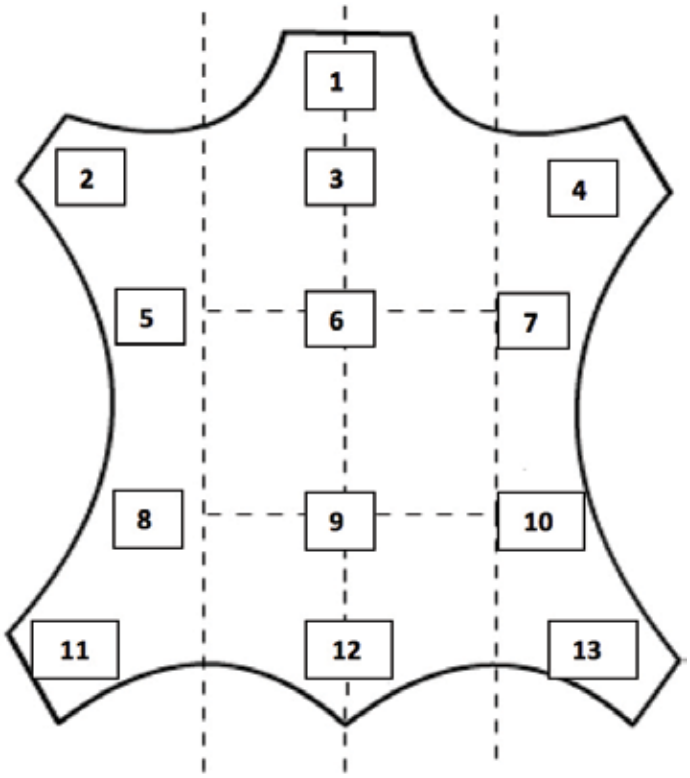

Figure 5. Sampling location on a whole skin.

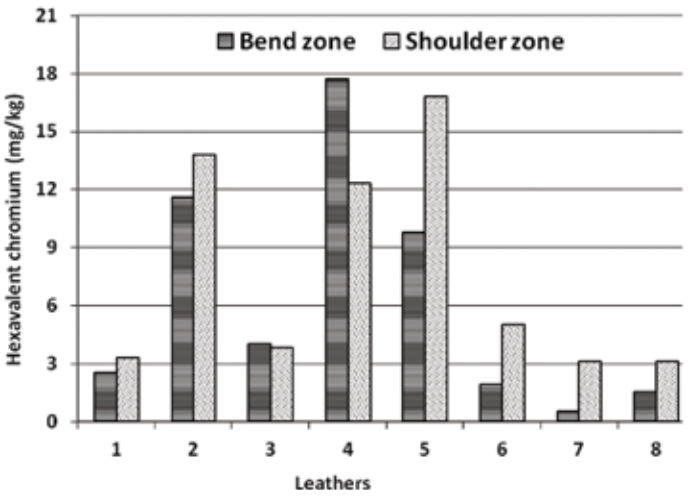

Figure 6. Influence of the sampling location in several leathers.

Although the standard ISO 2418:2002 is not applicable to leathers derived from birds, fishes or reptiles, the homogeneity of crocodile leather was evaluated in this study. The results, Figure 8, show that the only zones contaminated by hexavalent chromium are the extremities of the reptile leather. Indeed, only the crocodile head and legs contain more than 3 mg/kg of hexavalent chromium. The structure of collagen fibers of these zones may also be incriminated. The same tests will be realized on other types of skin as sheep and pig leathers. Preliminary results do not show a heterogeneous concentration of hexavalent chromium in these leather types but these findings require further investigation.

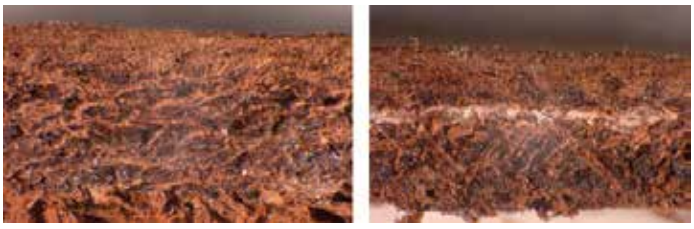

Figure 7. Comparison of fibers structure of collagen between the shoulders zone (left picture) and the bend zone (right picture) in a same leather. Optical microscope images of magnification 46x.

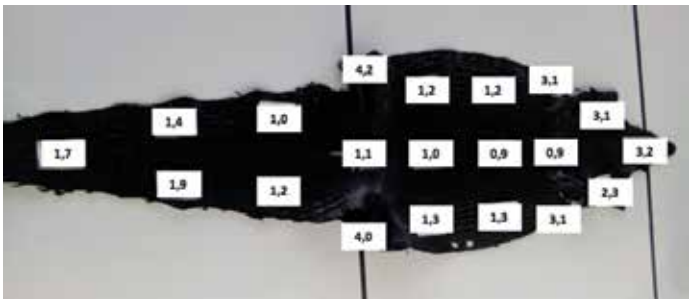

Figure 8. hexavalent chromium content measured in different sampling zones in same reptile leather (mg/kg).

Conclusion

It has been demonstrated that ion-chromatography with post-column derivatization can be implemented directly on crude extracts (without any pre-treatment) if a high capacity reversed-phase guard column is inserted before the analytical column. It allows the quantitative removal of co-extracted dyes, thus avoiding the poisoning of the analytical column and the degradation of its performances. The efficiency of this guard column remains total during more than 150 successive analysis of charged extracts with only a daily regeneration. The combination of the purification, separation and derivatization steps offer a number of benefits in terms of selectivity and reliability for the determination of hexavalent chromium in leathers in less than 10 minutes with minimal human handling. The method has been successfully validated according to the French standard NF-T90-210 with a presupposed LOQ of 5 µg/L corresponding to 0.25mg/kg of hexavalent chromium in the leather. Beyond the validation, this paper also highlights the crucial influence of the sample preparation ("grinding" or "cutting") on the hexavalent chromium content measured: the higher the division state of the leather, the higher the hexavalent chromium content measured. Cutting the leather even in very small pieces instead of grinding it, leads to an underestimation of hexavalent chromium. Considering the spatial distribution of hexavalent chromium in whole skins, the zones with less dense and more flexible collagen fibers seem to favor the oxidation of trivalent chromium into hexavalent one.

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