

DETERMINATION OF SOLUBLE HEXAVALENT CHROMIUM IN DYED CONSUMER GOODS IMPROVED BY A SIMPLE ON-LINE COLOR REMOVAL

by

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ABSTRACT

Hexavalent chromium detection in consumer articles is a statutory requirement and screening it in dyes and dyed leather is a challenge. Although many new improvised procedures were put forward, the lack of simplicity and automation leaves the void. The present study addresses these issues and offers a procedure that is closer to automated approach leading to finding that without the requirement of any special device the regular liquid chromatographic instrument can support a quick, simple and efficient method for hexavalent chromium detection in strongly colored leathers. The method was found to be effective in the removal of wide range of dyes and serving the detection of hexavalent chromium in ppb levels with %RSD of max. 3.5.

INTRODUCTION

Carcinogenic nature of hexavalent chromium is widely reported¹⁻³ and hence its detection even in trace levels is significant. The absence of hexavalent chromium is emphasized in water, food, waste water and in recent times also in consumer goods.⁴⁻⁶ Detecting soluble hexavalent chromium in water-soluble dyes and dyed consumer goods pose challenge for test houses when colorimetric procedure is followed. Official protocol⁷ is unable to serve with the reliability for such samples and its shortcomings are reported.^{8,9}

Chromium exists in multiple oxidation states and requires careful approach in sample preparation (especially while detecting in trace levels) as conditions in a method can facilitate conversion of one into another. In general, photometric determination through derivatization is a specific detection for an analyte and its selectivity is next best only to that of mass spectrometry. Samples like dyed leathers check these photometric techniques done even through selective derivatization of ChromiumVI for the reason that the presence of dyes does the intensity stealing.

There are methods available with good specificity to determine Cr (VI) in presence of Cr (III),¹⁰⁻¹⁴ but when high concentration of anionic dyes are present in the sample they adversely affect the chromatography and the detectors, more so if it is a photometric detector as reported in our earlier studies.¹⁵ Cr (VI) derivatization by diphenyl carbazide (DPC) serves as a very specific determination for Cr (VI) by photometry¹⁶⁻¹⁷ and the same reaction was used as post-column derivatization of an ion chromatography-photometry for the determination of Cr (VI)¹⁸ and the latter technique was rated to be several folds higher in sensitivity in comparison to the same by spectrophotometry as stated by NIOSH.¹⁹ As the Cr (VI)-DPC reaction leads to the formation of red to violet colored derivative absorbing at 540nm, the background of a colored matrix is undesirable.

Oxidative approach followed to quell organics in analytical methods for trace level determination of heavy metals has a number of shortcomings like long time duration, pollution, not being an on-line technique and the most importantly affecting the original oxidation states of the analyte and are the reasons that this approach is not possible for testing leather products. Hence, a technique free of hot conditions and oxidizers is desirable and the proposed technique meets the demand as it serves to clean the matrix interferants like soluble dyes.

Interference of water soluble dyes and their removal was reported in some earlier studies;^{8,9,20-23} two approaches were based on Solid-Phase Extraction (SPE) when one employed C₁₈,²⁰ the other employed florisil.²¹ The C₁₈ SPE approach is not effective against all shades of dyes and having limitation in capacity for dye concentrations and so is not dependable. The florisil SPE is effective but an off-line sample preparation technique. Scancar et.al. evaluated these two techniques and reported that florisil had been more effective for the dye removal while achieving good recoveries of the analyte.²² Recently, Wang Li et.al. reported²³ a flow injection-photometry involving Cr-DPC derivatization technique by studying different cartridge materials for color removal that included florisil for the

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Manuscript received December 15, 2014, accepted for publication March 11, 2015

determination of hexavalent chromium. That study employed column switching valves to do dye removal of the sample and then to reroute the sample to derivatization and detection of hexavalent chromium analyte. But, even Cr-DPC reaction method suffers from some interference²⁴ and hence direct photometry or flow injection technique might suffer whereas a chromatographic separation can clear those interference and assure a reliable value for Cr (VI). An on-line membrane based dialysis for removal of dyes followed by Ion chromatography-post column Cr-DPC reaction with photometric detection technique was reported for estimating Cr (VI) in waste water¹⁵ and in leather.²⁵ The on-line dialysis is a specialized approach for one to master with a considerable effort and hence may not suit every lab.

All these issues led to develop this simple sample clean up with accurate determination of Cr (VI) in consumer goods and also this approach is a step towards automation.

EXPERIMENTAL

Instrumentation

Ion chromatograph system of model 850 Professional IC pump, 887 Professional UV-VIS detector driven by MagIC Net software version 2.3, post-column reactor along with a peristaltic pump ISMATEC model "Ecoline" of IDEX corporation were all supplied by Metrohm AG, Herisau, Switzerland. Anionic IC column of Hamilton PRPX100 of 125x4mm with 10 μ m particle size was procured from Hamilton, Nevada, USA. Guard column hardware kit was of "Supelco" make of dimension 5cmx4.6mm i.d. The kit consists of a Guard column, 2 frits (2 μ m pores) and s.s.tubing of dimension of 2"/5cm of 0.01" i.d. x 1/16" o.d. and was procured from Sigma-Aldrich Corporation, St. Louis, MO, USA. Water for chromatography was prepared by using Millipore model Milli-Q Reference Q-POD Element, which was procured from Merck-EMD Millipore Corporation, Billerica, MA, USA.

Chemicals and Reagents

All the reagents were of analytical grade purity and sourced from Merck India, Mumbai. Florisil which is magnesium trisilicate, is available in two grades one was Supelco part No.2-0281 of particle size 74-149 microns (considered fine to coarse) and the other one was LC-Florisil of Supelco part number 5-7209 of particle size 125-149 microns (considered coarse), were solid-phase extraction bulk materials procured from Sigma-Aldrich Corporation, St. Louis, MO, USA. Potassium dichromate of Certified Reference material of ISO purity was procured from Merck, Darmstadt, Germany.

Standards Preparation

Using potassium dichromate of certified purity a 1000ppm stock solution was prepared using water of LC grade and from this, working standard solutions of Cr (VI) in the concentration range 10-100ppb were prepared afresh on daily basis.

For spiking studies, standard solution of 100 μ g ml⁻¹ was prepared afresh in water from the stock and was injected directly into the weighed sample taken separately for each trial by means of a Hamilton microlitre syringe as per the following: (i) for achieving 0.5mgkg⁻¹ spike level, 1 μ g was spiked by adding 10 μ l; (ii) for achieving 3mgkg⁻¹, 6 μ g was spiked by injecting 60 μ l; and for achieving 5mgkg⁻¹, 10 μ g was spiked by injecting 100 μ l.

Ion Chromatographic Conditions

50mM of ammonia-ammonium sulphate buffer of pH 7.5-8.0 was used as the mobile phase for the elution at isocratic flow rate of 0.3ml min⁻¹.

Preparation of Post-column Reagent

0.5g of 1,5-diphenylcarbazide was dissolved in 10ml of methanol LC grade, 25ml of orthophosphoric acid was added and made up to 1 liter with LC grade water.

Preparation of Guard Column

Florisil was used for packing the guard column of dimension mentioned. Florisil was made into slurry in methanol and filled into the column under vacuum with care taken to avoid any void formation. The guard column after packing, before connecting it to analytical column was subjected to thorough washing initially with water and then by mobile phase for conditioning.

Preparation of Buffer for Sample Extraction

Dipotassium hydrogen phosphate trihydrate was used for buffer by dissolving 22.8g in 1 liter of DI water. The buffer was used at pH of 8.0 \pm 0.2.

Preparation of Samples

Dye samples were directly dissolved in extraction buffer whereas leather samples were extracted with the same buffer as per IUC18/IULTCS 17075.⁷ The procedure in brief is discussed here. Leather samples were shredded to 100-200 microns size and about 2 g was weighed, transferred into a 250ml amber colored glass container with lid. To the sample, 100 ml of buffer was added, purged with inert gas, Argon and extraction was done using an orbital shaker at gentle oscillations of 50-60rpm. The extraction was done for 3 hours, the contents were filtered and taken immediately for analysis.

Procedure for Analyzing Samples

Sample prepared as detailed earlier was filtered through 0.45 μ m filter and 100 μ l was injected into the IC system. The guard column of choice was the one using florisil of the category fine to coarse. The ammonia-ammonium sulphate mobile was set at 0.3ml min⁻¹. The post column reagent was set at a flow of 0.5ml and the photometric detector was set at 540nm. After every sample or standard run completed, thorough washing with mobile phase was done before next one was injected.

RESULTS AND DISCUSSION

Different Grades of Florisil and Their Impact on Chrome Speciation and Dye Removal

The impact of florisil on chromium speciation was discussed earlier,^{21,22} which ruled out any conversion of oxidation states of chromium. The degree of removal was almost comparable for the two different grades of florisil. Dye removal with florisil was reported earlier²¹⁻²³ and this study apart from agreeing with those reports, found that even on dynamic conditions florisil remained effective. Even if column switching valves was reported²³ for on-line dye clean up that is not totally a dynamic condition as one end is a stagnating point where as this proposed study was a first dynamic dye removal study for analytical purpose to the best of our knowledge. Incidentally florisil is found to be effective in serving on-line cleaning up of such matrices for liquid chromatography techniques. Dye removal was found to be near total in each case of different shades of dyes studied; it was found to be 99.87% for the coarse one when it was 99.97% for the mixed type of fine to coarse. The results are produced in Table I.

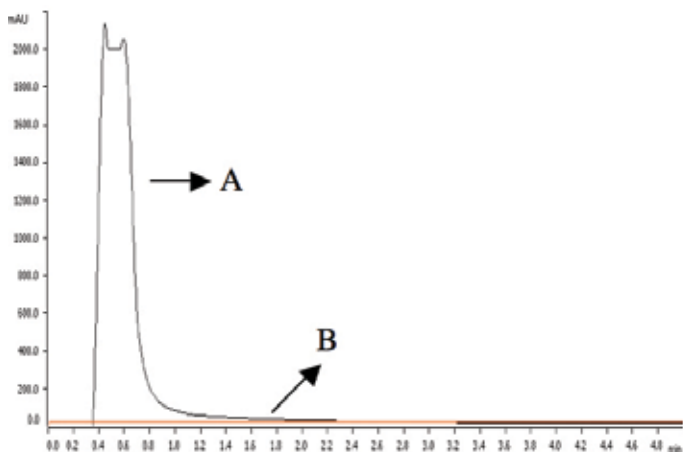


Figure 1. Comparison of dye removal without (A) & with (B) Florisil.

TABLE I
Comparison of dye removal by different grades of florisil.

TYPES OF FLORISIL	REMOVAL OF DYE (%)	
	1 st INJECTION	20 th INJECTION
FLORISIL (fine to coarse)	99.97	99.95
FLORISIL (coarse)	99.87	99.74

The Efficiency of Color Removal by Florisil

Studies from flow injection mode done with florisil guard column in place produced results for dye removal in the range 99.1-99.9% as compared to the one without florisil and the same is produced in Figure 1. Results following the same trend was found for various shades of dyes studied through the two different grades of florisil individually.

Tolerance Level of Dyes for Florisil Guard

This study was done to find out if it was necessary to change the florisil guard from run to run or on what frequency. A sample that was generating the most intensely colored extract of the lot consisting of scores of samples analyzed for Cr (VI), was chosen for this study. That chosen sample's analytical extract when analyzed by spectrophotometer (with out any addition of DPC to derivatize) to ascertain only the response of the dye, was found to have wavelength maximum of 530nm and the absorption at the analytical wavelength (540nm) was found to exceed the value of 2 even on 20 times dilution proving that sample is a strongly dyed type. The fresh extract of the same sample without any dilution was injected repeatedly through the same florisil guard packing in flow injection mode till the bleeding of dye would result. But nearly 30 injections were completed the bleeding was not noticed. Earlier, the original concentration of dye was recorded without florisil in place against which the dye removal was compared. The dye removal was at around 99.8% at the beginning and the same level of removal was recorded after 30 injections. However at this point backpressure developed enormously and hence any further injection was not possible. The Figure 2 is furnished to show the status of the dye removal by florisil after 20 repeated injections and at this stage the back pressure was also under control.

When Cr (VI) was spiked at 6 μ g to 2g of sample (that was found to contain not any detectable concentration of Cr (VI) by this proposed procedure) and subjected to analysis, the recovery found at this stage of florisil guard column was 99.1%.

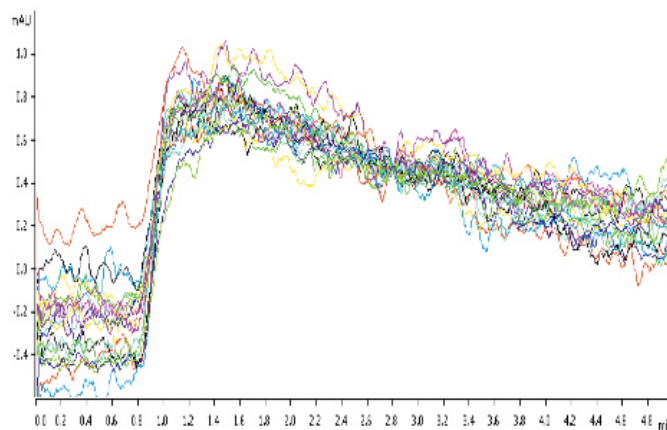


Figure 2. Efficiency of dye removal by Florisil studied in flow injection mode; evidencing for remaining effective in dye removal even after 20 repeated injections.

This study carried out proved that florisol guard column served reliable for dye removal and for the recovery of Cr (VI) even on multiple uses of as many as 20 injections of intensely dye carrying samples.

Recovery of Hexavalent Chromium

Like dye removal studies this study was also carried out without column in place .The recovery studies were attempted by injecting standards of 10-100 ppb ($\mu\text{g l}^{-1}$) directly by employing the two different florisol grades in separate attempts to ascertain if particle size of florisol could impact the recovery of Cr (VI). The findings revealed that the recovery varied with concentration range of analyte, as it was 86.9% in the case of coarse and 94.9% with finer to coarse one, for the lowest concentration level of 10 ppb in this study. The results for the concentrations in the range 20-100 ppb were found to be in the range 95-98.5% for coarse one and 96-99.1% for the fine-coarse type. The detailed results are produced in Table II.

TABLE II
Comparison of recovery of Cr (VI) by florisol (fine to coarse) and florisol (coarse).

Concentration of STD (in $\mu\text{g l}^{-1}$)	Recovery (%)	
	LC-florisol, (Fine to coarse)	Florisol, (coarse)
10.0	94.9	86.9
20.0	96.0	95.2
60.0	98.6	98.2
100.0	99.1	98.5

TABLE III
Recovery studies done with two samples by finding the original and spiked concentrations of Cr (VI) and their comparison with the official IUC-18 method.

Nature of sample	Color of the extract	Content of Cr(VI)									
		Proposed method involving Florisol guard column IC post-column derivatization Photometry.					Official IUC -18/ISO 17075				
		Amount originally found (mg kg^{-1})	Spiked level (mg kg^{-1})	Total amount (mg kg^{-1})	Recovery (%)	%RSD(from 6 replicates)	Amount originally found (mg kg^{-1})	Spiked level (mg kg^{-1})	Total amount (mg kg^{-1})	Recovery (%)	%RSD(from 6 replicates)
Leather foot wear (children wear)	Purple	1.40	----	1.40	----	2.0	2.32	----	2.32	----	5.9
			0.50	1.89	99.5	1.8		0.50	5.33	189.0	11.5
			3.00	4.37	99.3	2.3		3.00	7.09	133.3	14.1
			5.00	6.49	101.4	2.5		5.00	8.35	114.1	19.2
Leather watch strap (Beige colored)	Color-less	Non detected	----	----	----	1.1	Non detected	----	----	----	1.8
			0.50	0.49	98.0	2.3		0.50	0.44	88.0	2.4
			3.00	3.10	103.3	1.9		3.00	3.05	101.7	2.1
			5.00	4.92	98.4	2.4		5.00	4.87	97.4	2.6

The results led to the choice of florisil of finer to coarse grade for this study as it could support the recovery of Cr (VI) even in low concentrations.

Choice of Flow Rate of Mobile Phase

Flow rate in the range of 0.6-1.2 ml was helpful in this analysis to achieve a quick elution but it was only for the initial stages of florisil guard column and once when 10 samples injected the pressure slowly started building up and at the completion of about 20 samples the system tripped down. Only after a wash with 10% aqueous methanol for about 20 column volumes, it was possible to restore the analysis. This led to the choice of 0.3 ml of flow rate for the analysis right from the first sample till the twentieth.

Validation of Method

Spiked studies of samples with hexavalent chromium were done at levels 0.5mg kg^{-1} , 3mg kg^{-1} and 5mg kg^{-1} following the spiking procedure detailed earlier. In which case, 3mg kg^{-1} represents the maximum permissible limits by the European legislation. The leather chosen was that which generated intensely (purple as that exactly absorbs at 540nm with absorption value showing 2.5 for 15 times dilution of the extract) colored extracts so that effects of dye removal, any interference due to residual dye levels if encountered and the recovery of hexavalent chromium all could be accounted. The results are produced in Table III along with the values for reproducibility of the proposed method. The results revealed that the results of the sample (foot wear) producing purple colored extract obtained from florisil – IC photometric and the Official method did not agree, the results from official method is about 66% higher than the proposed procedure and also with poorer reproducibility. The outcome of the spiked results also followed the same pattern as florisil IC produced results for the spiked samples matching exactly the calculated values, falling in the recovery range of 99.3-101.4% while the results from the official method were erratic and with higher RSD. In the next case, a watch strap, which generated a colorless extract, results from both the methods were almost in agreement with good reproducibility. These observations helped to confirm that the results generated by the official method suffered badly because of interference of dyes remained substantially beating C_{18} SPE of the official method. In contrast, the method proposed ensured good recovery values through out with good reproducibility for the same matrices and that is an important indication for compliance of validity experiments.

Analysis of Real Samples

Samples of dyes and consumer goods of leather (shoes, watch strap, belts and handbag) and a cotton textile were analyzed for the hexavalent chromium by adopting the extraction method of IUC-18 and then the extracts were analyzed by florisil cleanup-IC photometric analysis after filtration. There after, the spiked studies were done by adding standard Cr (VI) solution equivalent to $6\mu\text{g}$ to each of those samples of 2g weight, directly and analyzed by the proposed method. This

level of spiking was done for the reason aforesaid. The results for real samples analyzed by the proposed procedure ranged from “Not detected” to 4.03 with %RSD in the range 1.1 to 2.8 as maximum and only one sample of this set was found to exceed the European permissible limit of 3mgkg^{-1} . In the case of spiked samples also recovery ranged from 97.8-103.3% with %RSD of maximum 3.5. In contrast, the official method produced results of almost double and in some cases even 5 times the value of the proposed method with %RSD in the range of 2.1 to 17.2 and for spiked cases with erratic recoveries evidenced by poor %RSD values. However, the results from both the methods were almost in agreement only for 3 samples listed last in the Table IV and the agreement prevailed even for their spiked cases. The results for Cr (VI) detected in real samples were furnished in Table IV. The chromatograms of some of these studies are produced as Figures 3 and 4. In Figure 3, Cr (VI) found originally in a red to violet colored sample extract and its spiked recovery are shown while in Figure 4, the original level of Cr (VI) found in a pink colored extract is shown. Mean while the results from the official method for the cases of colorless extract, orange colored extract of cotton and brown colored extract agreed well with the proposed method. The reason is that in the case of colorless extract of watch strap it is obvious that no interfering component is present while in the cases of orange colored extract of cotton (with absorption value of 0.6 at 540nm) and

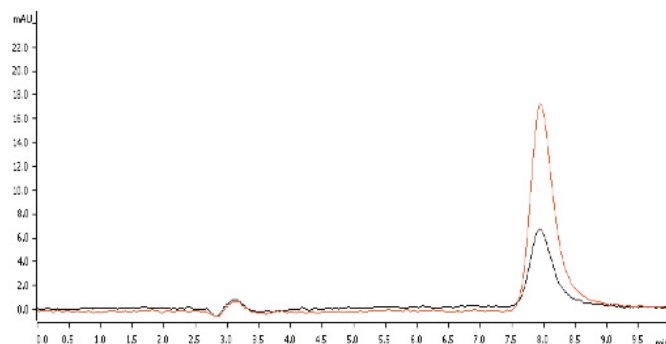


Figure 3. Cr(VI) detected in the extract of dark red to violet dyed leather with spike.

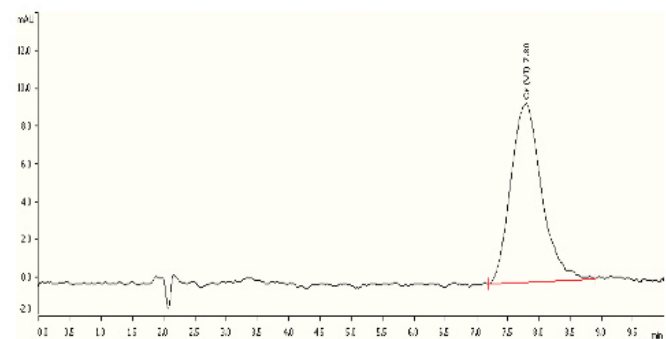


Figure 4. Cr(VI) detected in pink colored extract of leather sample.

brown colored extract of leather belt (with absorption value of 0.4 at 540nm), the color observed was very feeble in each of these cases and hence getting easily eliminated by C₁₈ SPE followed in the official method. The observed trend in the results is that the results from the proposed florisol- IC and the official methods agree whenever the dye is absent or

completely eliminated in the extracts ;otherwise, they disagree revealing the interference of dyes by the partially effective official method. The results revealed that the interference due to dyes in the photometric detection was completely overcome by this simple on-line approach and analysis of sample was completed with in 10 min.

TABLE IV
Comparison of Cr(VI) results for the real samples of consumer goods of various shades when analyzed by the official IUC/ISO and the proposed method.

Nature of sample	Color of the extract	Content of Cr(VI) (mg kg ⁻¹)				
		Type of Analysis	Florisol guard column IC post-column derivatization Photometry		Official IUC -18 /ISO 17075	
			Content of Cr(VI) (mg kg ⁻¹)	%RSD (from 6 replicates)	Content of Cr(VI) (mg kg ⁻¹)	%RSD (from 6 replicates)
Leather handbag Spike	Blue	As such	2.32	2.8	4.86	11.3
		+ spike*@3 (mg kg ⁻¹)	5.29	3.1	10.1	13.7
Leather Footwear	Red	As such	1.94	2.4	2.91	8.3
		+ spike*@3 (mg kg ⁻¹)	4.95	2.8	6.7	9.0
Leather footwear(children)	Purple	As such	1.43	2.1	2.3	14.1
		+spike*@3 (mg kg ⁻¹)	4.37	3.5	7.0	13.6
Leather watch strap	Black	As such	1.14	2.0	6.0	17.2
		+spike*@3 (mg kg ⁻¹)	4.20	2.5	4.5	21.4
Leather belt	Brown	As such	4.03	1.8	3.9	3.7
		+spike*@3 (mg kg ⁻¹)	7.11	1.6	6.8	4.6
Cotton gloves	orange	As such	2.06	2.1	1.9	3.1
		Spike*@3 (mg kg ⁻¹)	4.95	2.7	5.24	3.9
Leather Watch strap(beige)	colorless	As such	Not detected	1.1	Not detected	2.1
		Spike*@3(mg kg ⁻¹)	3.01	1.4	2.95	1.9

*Spike is done at 6µg to2g sample that is representing 3mgkg⁻¹.

CONCLUSION

Florisil based dye cleaning guard columns are promising for clearing the colorants in particular organic anionic dyes, thereby facilitating highly selective photometric detection of hexavalent chromium analytes. The scope can be expanded for the removal of colorants mainly dyes in photometric detections of various analytes.

ACKNOWLEDGEMENT

The authors thank CSIR for their support in this research study and this article bears Library reposit number of CSIR-CLRI commun.No.A/2014/EXL/CSIR-CLRI/1093.

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