Method Development for Analyzing Hexavalent Chromium in Water Soluble Dyes and its Validation

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Abstract

Hexavalent chromium present in water-soluble dyes is eluted through an activated carbon solid-phase extraction cartridge at a pH of 12 – 13. Dye chromophores, which interfere with the colourimetric detection of hexavalent chromium are removed by absorbing on the activated carbon bed. Un retained Cr(VI), eluted through activated carbon solid phase extraction system will give a purple-violet colour with Diphenylcarbazide reagent. Cr(VI) oxidizes 1,5 diphenylcarbazide to 1,5 diphenylcarbazone to give a purple /violet complex, which can be quantified spectrophotometrically at 540 nm wave length. The method described is suitable to quantify chromium (VI) content in water-soluble dyes down to 3 mg/kg. This in house developed test method has been validated as per international validation protocol for spike recovery at three different levels, repeatability (intra assay and intermediate precision), LOD, and LOQ for dyes like Reactive yellow HE 6G, Reactive red 218, Turquoise blue HGN, Reactive navy blue RX, Reactive black 5A.

Key Words:

Activated carbon, Diphenylcarbazide, Hexavalent chromium, Inductively Coupled Plasma-Optical Emission Spectrometry, Spectrophotometer, Solid phase extraction.

1.0 Introduction

In its natural state, chromium mainly occurs in its trivalent [Cr(III)] and hexavalent[Cr(VI)] forms. Trivalent chromium is an essential micronutrient for humans and is nontoxic in normal doses in the food supply; at higher doses, however, trivalent chromium can exhibit cytotoxicity. Due to its high solubility and oxidizing property, hexavalent chromium is extremely toxic and has been confirmed to have a carcinogenic effect [1]. It is a well-established fact that once Cr(VI) reaches the bloodstream, it damages the kidney, liver, and blood cells of the human through oxidations reaction, and the patient leads to hemolysis, renal, and liver failure [2]. Cr(VI) leads to allergic reactions in human beings and even at low concentrations it can causes diseases such as dermatitis, ulceration, encephalopathy, anemia, hepatitis, and nephritic syndrome [3-5].Cr(VI) also possesses carcinogenicity and can damage the DNA of humans and animals [6].

Cr(VI) has a wide range of applications in industrial fields. A large number of chromium-based synthetic dyes are being increasingly used in the textile, paper, pharmaceutical, cosmetic, and food industries. It is also used in electroplating, cement, steel, paint, dye, aluminum, leather tanning, metal finishing, and chromate manufacturing industries [7-8]. Thus, the effluents from these industries, when mixes with the soil or surface water create a health hazard to mankind. It is therefore needed to develop a method for environmental cleanup and remediation so that Cr(VI) is safely removed from the water. In the past few years, many techniques such as chemical precipitation, coagulation-flocculation, flotation, electrochemical, ultrafiltration, etc. have been used by various workers [9-12].

The total chromium content at trace levels is determined traditionally using graphite furnace atomic absorption spectroscopy (GFAAS) or by ICP OES.

The colourimetric method is widely used for the estimation of Cr(VI) in textiles, leather, drinking water, electronic components, etc.[13-15]. However, this method cannot be applied for the estimation of trace level of Cr(VI) in water soluble dyes, colored effluents, etc. due to the color interference while measuring the optical density of Cr(VI).

Simultaneous determination of trivalent and hexavalent chromium using accelerated solvent extraction and ion chromatography, LC-ICP-MS, etc. are some of the

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hyphenated techniques being used to analyze Cr(VI) [16-17]. Research work has also been carried out to separate Cr(VI) from water soluble dyes by HPLC pre-column as well as post column derivatization method [18-19]. However, all these instruments are very costly and many of the small-scale testing laboratories may find it difficult to invest a huge amount for costly instruments. Hence, it is the need of the hour to develop a simple and economically viable test method to quantify trace levels of Cr(VI) in water-soluble dyes.

In this study, we have explored various techniques to segregate Cr(VI) from dye chromophore, out of which, an activated charcoal bed was found to be the best absorption media for separation. The method involves a simultaneous process of absorption of dyes in an alkaline phase-activated charcoal and elution of hexavalent chromium.

2.0 Material and Method

2.1 Materials

- Pipette of capacities 1ml,10ml
- Glass beaker of capacities 10 ml
- Volumetric flask of capacities 25ml,100ml,1000 ml
- Spectrophotometer wave length 540 nm
- pH meter in the range of 1-14
- Vacuum manifold connected to vacuum pump.
- Solid Phase Extraction (SPE) Column (3.0 g of activated charcoal in a 5.0 ml capacity syringe column)

2.2 Reagents

- Diphenylcarbazide solution (1.0g 1, 5-diphenylcarbazide (DPC) in 100 ml acetone and acidified with one drop of glacial acetic acid.
- O-phosphoric acid solution (88%)
- Nitric acid (69%)
- Hydrofluoric acid (48%)
- Hydrochloric acid (37%)
- NaOH (3.0 % solution)
- Na_2CO_3 (0.5 % solution)
- Eluent mixture (50 ml 3% NaOH and 50 ml 0.5 % Na_2CO_3), pH 12-13
- Distilled water, Grade 3 quality as specified in ISO 3696
- Activated charcoal, MERCK (7440-44-0) particle size 100 mesh
- Potassium dichromate ($K_2Cr_2O_7$), dried for 16 hours at 102°C
- Cr(VI) stock solution: Dissolve 2.829 g $K_2Cr_2O_7$ (pre-dried for 16.0 hrs. at 102°C ±2°C) in distilled water and makeup to 1000 ml. 1 ml of this solution contains 1mg of Cr(VI) (1000 ppm).
- Cr(VI) standard solution: From stock solution pipette out 1 ml into a 100 ml volumetric flask and makeup to the mark with distilled water.1 ml of this solution contains 0.01 µg of Cr(VI) (10 ppm)

2.3 Instruments

- Analytical balance of accuracy 1 mg (Shimadzu AUX 220)
- pH meter, with a glass electrode(ELICO LI127)
- Microwave Digestion System (Anton Paar Multiwave GO)
- UV-VIS Spectrophotometer (Shimadzu UV 1800)
- Inductive Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) (Perkin Elmer Optima 8000)

2.4 Methods

2.4.1 Estimation of total chromium content in dyes

In principle, the trace metal content in any matrix can be estimated after being subjected to either conventional acid digestion or high temperature and high-pressure microwave-assisted digestion, depending on the nature of the matrix. The digested sample can be analyzed by AAS/ICP-OES to quantify the metals. To estimate the total chromium (Cr(III) & Cr(VI)) in dyes, a known weight of the sample was digested using 10ml nitric acid in a microwave digestion system. The digested sample was diluted up to a certain volume and the total chromium content was determined using ICP-OES.

2.4.2 Estimation of Cr(VI) in dyes

Since Cr can undergo a redox reaction, it can easily oxidize to Cr(VI) and can reduce to Cr(III), care should be taken while the digestion/extraction process. All oxidizing agents and reducing agents should be avoided during the extraction/digestion process. By keeping this in mind, we have made various trials to segregate the Cr(VI) from dyes. For this purpose, first, we have studied the effect of different acids and alkalis on Cr(VI) during the digestion/extraction process.

2.4.2.1 Conventional acid digestion method

A known concentration of Cr (VI) solution was treated with different acids and digested using a conventional hot plate digestion method. After digestion, the solutions were derivatized with diphenylcarbazide and o-phosphoric acid, and color was developed. The optical density of the solution was measured at 540nm using UV-VIS Spectrophotometer and compared against standard.

2.4.2.2 Microwave assisted acid digestion method

Microwave digestion techniques are used to digest the toughest sample, as it works at high temperature (200-250°C) and high pressure (20-40 bar). To study the impact of various acids at high temperature and pressure on Cr(VI), a known concentration of Cr (VI) solution was digested with various acids in a microwave digestion system. After digestion, the

solutions were derivatized with diphenylcarbazide and o-phosphoric acid, and colour was developed. The optical density of the solution was measured at 540nm using UV-VIS Spectrophotometer and compared against standard.

2.4.2.3 Alkaline digestion method

Literature survey reveals that Cr(VI) is stable at alkaline pH and standard method like EPA recommends alkaline extraction for estimation of Cr(VI) from the soil. However, the efficacy of alkaline extraction for the estimation of Cr(VI) from dyes has not been studied. To study the alkaline extraction efficacy of Cr(VI) from dyes, a known concentration of hexavalent chromium was taken in a 100 ml conical flask.10 ml of alkaline solution (2% of NaOH and 3% Na_2CO_3) was added to it. The solution was digested on a hot plate; then the solution was maintained at this temperature for 30mins. The flask was cooled to room temperature and the solution was derivatized using diphenylcarbazide and o-phosphoric acid and color were developed. The optical density of the solution was measured at 540 nm using UV-VIS Spectrophotometer and compared against standard.

2.4.2.4 Activated charcoal SPE method

- Preparation of SPE column

3.0 gram of activated charcoal powder (2.2.10) was weighed and filled in a 5.0 ml capacity syringe column. Before filling activated carbon powder, 4-5 layers of filter paper/nylon membrane were added to support the fine carbon particles in the column. An activated charcoal column was connected to a vacuum manifold (2.1.6). The column was washed 2-3 times with an eluent mixture (2.2.8) under vacuum.

- Analysis of Cr(VI) standard solution

1.0 ml of Cr(VI) solution (2.2.13) was loaded into a pre washed activated charcoal solid phase extraction cartridge and allowed to absorb in the column. Cr(VI) was eluted from the column with 20.0 ml of eluent mixture (2.2.8). The extract was collected in a 25.0 ml volumetric flask and colour was developed with 1.0 ml phosphoric acid (2.2.2) and 1.5 ml DPC solution (2.2.1). The final solution was made up to the mark with distilled water. The absorbance of the solution was measured at 540 nm wave length by using a spectrophotometer. The absorbance of this solution will be equivalent to 0.4 ppm concentration of Cr(VI).

- Analysis of dyes

0.5 g of dye was weighed in a clean glass beaker to the nearest 0.001g. The dye was dissolved in a 10 ml eluent mixture (2.2.8) and a stock solution was prepared.

The above entire stock solution was loaded directly into a pre washed SPE column (2.1.7) drop by drop and the solution was allowed to absorb throughout the column. The beaker was washed with a 5ml eluent mixture (2.2.8) and the solution was poured drop by drop on top of the column and Cr(VI) was eluted under vacuum. The extraction was repeated another 3 times with a 5.0 ml aliquot of eluent mixture. The entire extract (approximately 20ml) was collected in a 25.0 ml volumetric flask. (Dye molecules will absorb in activated carbon under alkaline pH and unretained Cr (VI) will elute). The color was developed by adding 1.0 ml o-Phosphoric acid (2.2.2) followed by 1.0 ml 1,5 DPC solution (2.2.1) and the solution was made up to 25.0 ml with distilled water. This solution was kept for 5 minutes at ambient temperature and the absorbance was measured at 540 nm wave length by using a Spectrophotometer against a blank solution.

Note: The above weight of dye and volume of the extract is for the determination of Cr(VI) down up to 3.0 mg/kg. If the Cr(VI) content is high, either a less weight or a small aliquot from the stock solution may be taken so that absorbance value is within the calibration range of Cr(VI).

- Blank solution

The eluent mixture (2.2.8) was filled three quarters in a 25ml volumetric flask and 1.0 ml phosphoric acid (2.2.2) followed by 1.0 ml 1,5 DPC solution (2.2.1) was added and makeup to 25 ml.

2.5 Calculation

Calculate the Cr(VI) content in dyes as per the given formula

$$wCr(VI) = \frac{C \times V}{m}$$

Where,

wCr(VI) is the mass fraction, expressed in milligram per kilogram (ppm) of soluble Cr(VI) in dyes.

C is the concentration of Cr(VI) obtained from the calibration graph of standard (mg/kg)

m is the mass of the dye sample taken, expressed in grams (g)

V is the dilution factor (ml)

3.0 Results and Discussion

Total chromium content in various commercial dyes analyzed by ICP OES is tabulated in Table 1. It has been observed that few dyes like Direct blue GL, Reactive Black, Basic Brown, etc. do not contain any chromium, whereas, dyes like XP-20 N, disperse black, etc. contain a high amount of chromium. The total chromium content in XP-20N is 1.55% and disperse black is 0.15%. Since these are total chromium (Cr (III) and Cr(VI)), a good separation technique is needed to segregate and estimate hexavalent chromium.

Table 1 Total chromium content in Dyes

Dyes	Chromium (mg/kg)
Direct blue GIL	ND
Reactive black	ND
Remazole black	ND
Basic brown	ND
Solvent brown	ND
Solo blue B	ND
Solo blue T	ND
Reactive orange	4.0
XP-20 N	15570
Disperse black	1556

Various acids are used for the digestion of Dyes to estimate total chromium by conventional as well as microwave digestion methods. But the effect of these acids on Cr(VI) under various temperatures and pressure has not been studied. Hence, we have conducted a study on the effect of acids on Cr(VI). Table 2 shows the effect of various acids on hexavalent chromium under conventional digestion.

From Table 2, it was observed that digestion of Cr (VI) by a conventional method is not suitable, as all the acids interfere either with Cr (VI) or with DPC. When hexavalent chromium was digested by a conventional method, without acid (only with water) purple/violet color of Cr (VI) developed and the color was stable for a long duration. This indicates that most of the acids have an adverse impact on Cr(VI).

Table 2 - Effect of various acids on Cr(VI) in a Conventional digestion

Conc. Of Cr (VI)	0.4mg/kg	0.4mg/kg	0.4mg/kg	0.4mg/kg
Acids	1ml HNO_3	1ml HCl	1ml HF	1ml Water
Method	Digestion in hot plate followed by colour development			
Visual Observation	The purple colour of Cr(VI) is not Stable	The purple colour of Cr(VI) is not Stable	The purple colour of Cr(VI) is not Stable	Purple colour of Cr(VI) Stable

Table 3 shows the effect of various acids on Cr(VI) at high temperature and high-pressure microwave digestion system.

When a Standard solution of Cr(VI) was digested in a microwave digestion system without any acid (only with water) purple color of Cr (VI) developed and the color was stable for a long duration. At the same time, when the same standard solutions were treated with various acids and derivatized with DPC, the color was either not developed or the developed colour was not stable. The same effect was observed in a hot plate conventional acid digestion also. This indicates that no acids can be used for the analysis of Cr(VI) as it adversely affects the optical density measurement.

Table 3 - Effect of acids on Cr(VI) by Microwave digestion system

Conc. Of Cr(VI)	0.4mg/kg	0.4mg/kg	0.4mg/kg	0.4mg/kg
Acids	9ml HNO_3	9ml HCl	9ml HF	9ml Water
Method	By Microwave Digestion followed by colour development			
Visual Observation	No Purple colour	No Purple Colour	No Purple colour	Purple colour Stable

Table 4 shows the effect of alkaline extraction on Cr(VI) based dyes. The results indicate that hexavalent chromium can be extracted with the alkaline digestion method with a recovery of 90%. However, this method is not suitable for analysis of Cr(VI) in water soluble dyes as in alkaline extraction the color of the dye (chromophore) will also co elutes along with Cr(VI).

Since hexavalent chromium is having good stability at alkaline pH, few extraction trials were conducted in an alkaline medium. It was found that activated charcoal, at an alkaline pH can absorb all types of water soluble dyes, at the same time it doesn't have any affinity to Cr(VI). Based on this finding, a study was carried out by spiking a concentration of 5.0mg/kg standard Cr(VI) in various water soluble dyes which were free from chromium. The dye solution spiked with Cr(VI) was loaded into an activated carbon bed at pH 12 to 13. The substance which interferes with the detection (dye chromophores) is removed by absorbing in the activated carbon bed and unretained Cr(VI) was eluted. The resultant eluent was derivatised and the developed color was measured in a UV VIS spectrophotometer at 540nm wavelength. The spike recovery thus obtained is tabulated in Table 5. The Recovery of Cr(VI) was found to be more than 90 % in all dyes. This indicates that estimation of Cr(VI) by activated carbon in alkaline pH can be used to segregate hexavalent chromium from interfering dye chromophores. Based on the spike recovery study, further, the method has been validated as per the International validation protocol.

Table 4 Effect of Alkalis on Cr(VI) based dyes

Samples	Standard Cr(VI)	Reactive Black dyes spiked with Cr(VI)	Basic Brown spiked with Cr(VI)
Cr(VI) Concentration	0.4mg/kg	0.4mg/kg	0.4mg/kg
Method	Alkaline digestion followed by colour development		
Visual observation	Purple colour	Co-elution of dye colour (Black)	Co elution of dye colour (Brown)
Measured Concentration	0.360 mg/kg	Measurement not possible	Measurement not possible
Recovery (%)	90	-	-

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Water soluble dyes	Spiked Conc. (mg/kg)	Replicates of Recovered Concentration(mg/kg)						Recovery (%)
		1	2	3	4	5	Mean	
Yellow HE 6G	5.0	4.92	4.64	4.88	4.76	4.88	4.81	96.2
Reactive Red 218	5.0	4.80	4.88	4.73	4.64	4.80	4.77	95.4
Reactive black 5A	5.0	4.60	4.72	4.50	4.57	4.68	4.61	92.2
Navy Blue RX	5.0	4.60	4.84	4.58	4.53	4.76	4.66	93.2

4.0 Method Validation

The developed test method has been validated as per Eurachem and ICH guidelines for the determination of Accuracy, Precision, Reproducibility, Linearity, LOD & LOQ.

4.1 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. For the quantitative approaches, accuracy can be determined by spiking three levels and at least 10 replicates should be obtained. The percentage recovery or the difference between the mean and the accepted true value together with the confidence levels are recommended. For accuracy, the mean recovery within 90-110% of the theoretical value may be taken as acceptance criteria.

To determine the spike recovery, a known amount of three different concentrations i.e. 5mg/kg, 10mg/kg, 20mg/kg of Cr(VI) standard solution was spiked in five different dyes. The spiked samples were analyzed as per the test method and the spike recovery was calculated. Ten replicates were taken to calculate the statistical parameters like SD and RSD. The results are tabulated in Table 6A, 6B, & 6C.

From Table 6A it can be seen that the Spike recovery of all dyes is above 90%. Similar spike recovery is shown in 10 mg/kg and 20 mg/kg levels also. The percentage relative standard deviation (RSD) of recovery is below 5.0%.

Table 6A Spike recovery of Cr(VI) at 5 mg/kg

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Dyes #	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy blue RX	Reactive Black 5A
1	98.4	96	98.4	92	92
2	92.8	100	96.8	96.8	94.4
3	97.7	94.6	96.9	91.6	90.2
4	95.3	96	97.6	90.6	91.4
5	97.6	92.9	88.9	95.2	93.7
6	94.6	97.6	93	96.1	91.5
7	96.1	91.6	93.1	96.1	90
8	91.4	93.7	96.8	99.2	96
9	95.3	92.9	90.6	96	93.7
10	89	100	95.3	92.9	98.4
Mean (%)	94.82	95.53	94.74	94.65	93.13
SD (ppm)	2.99	2.95	3.20	2.74	2.66
RSD (%)	3.16	3.09	3.37	2.89	2.85

Table 6B Spike recovery of Cr(VI) at 10 mg/kg

Dyes #	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
1	92.3	93.1	90.7	93.5	94.3
2	94.4	93.8	86.1	98.3	95
3	94.7	97.1	97.9	98.3	95.1
4	97.7	102	95	93.6	97.7
5	95.4	94.1	90	90	90.5
6	86.7	92.6	93.7	93.3	99.2
7	96.1	94.5	91.8	91.8	93.3
8	91.2	94.4	95.2	95.6	94.4
9	96.8	96	94.1	90.9	91.7
10	98.4	92.4	93.6	96.8	98.8
Mean(%)	94.37	95	92.81	94.21	95
SD (ppm)	3.50	2.86	3.30	2.95	2.87
RSD(%)	3.71	3.01	3.55	3.13	3.02

Table 6C Spike recovery of Cr(VI) at 20 mg/kg

Dyes #	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
1	97.2	92.1	90.5	91.3	90.5
2	93.9	94.3	91	93.5	98.3
3	99.2	90.1	95.6	93.6	90.9
4	89.8	94.9	87.1	86.7	87.9
5	95.5	91.5	91.9	93.9	91.9
6	95.5	91.2	97.5	98.3	94.3
7	96.4	90.9	90.5	92.9	94.1
8	96.1	94.2	92.2	91.4	90.6
9	94.9	94	97.6	99.6	98.8
10	97.4	94.6	90.5	98.6	95.9
Mean (%)	95.59	92.78	92.44	93.98	93.32
SD (ppm)	2.51	1.79	3.41	3.94	3.58
RSD(%)	2.63	1.93	3.69	4.20	3.84

4.2 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision (measurement precision) is a measure of how close results are to one another. It is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation (or relative standard deviation) calculated from results obtained by carrying out replicate measurements on a suitable material under specified conditions. Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility.

4.2.1 Repeatability (Intra- assay precision)

Repeatability, expected to give the smallest variation in results, is a measure of the variability in results when a measurement is performed by a single analyst using the same equipment over a short timescale. Repeatability expresses the precision under the same operating conditions over a short interval of time. To determine the repeatability, a known amount of Cr(VI) was added to five different dyes. This test was carried out for six different days and the mean, SD, RSD was calculated. The results are tabulated in Table 7

Table 7 Repeatability of Cr(VI) in dyes at 10 mg/kg

Dyes	Yellow HE 6G	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
Days					
Day-1	9.23	9.31	9.07	9.35	9.43
Day-2	9.44	9.38	8.61	9.83	9.5
Day-3	9.47	9.71	9.79	9.83	9.51
Day-4	9.77	10.2	9.5	9.36	9.77
Day-5	9.92	9.68	9.02	8.91	8.25
Day-6	9.12	9.44	9.52	9.56	9.44
Day-7	9.79	9.79	9.38	9.58	9.54
Mean (mg/kg)	9.53	9.64	9.27	9.49	9.35
SD(ppm)	0.30	0.31	0.39	0.32	0.50
RSD(%)	3.17	3.16	4.26	3.38	5.32

From Table 7, the data shows that the RSD % of repeatability of all dyes is below 5% which is within the acceptable limits.

4.2.2 Intermediate precision

Intermediate precision expresses within laboratories variation: different days, different analysts, different equipment, etc. Intermediate precision gives an estimate of the variation in results when measurements are made in a single laboratory but under specified conditions that are more variable than repeatability conditions. The aim is to obtain a precision estimate that reflects all sources of variation that will occur in a single laboratory under routine conditions (different analysts, extended timescale, etc.)

To determine the intermediate precision, a known amount of Cr(VI) was spiked in five different dyes. Cr(VI) in these dyes was measured on different days by seven different analysts and the mean, SD, RSD was calculated. The results are tabulated in Table 8.

Table 8 Intermediate precision of Cr(VI) at 10mg/kg

Operator	Dyes	Yellow HG 6E	Reactive Red 218	Turquoise Blue HGN	Navy Blue RX	Reactive Black 5A
	Days					
A	Day-1	9.47	9.71	9.79	9.83	9.51
B	Day-2	9.54	9.41	9	9	9.05
C	Day-3	8.67	9.26	9.37	9.33	9.92
D	Day-4	9.61	9.45	9.18	9.18	9.33
E	Day-5	9.68	9.6	9.41	9.09	9.17
F	Day-6	9.84	9.24	9.36	9.68	9.88
G	Day-7	9.51	9.27	9.35	9.47	9.23
	Mean	9.47	9.42	9.35	9.37	9.44
	SD(ppm)	0.38	0.18	0.24	0.31	0.34
	RSD(%)	3.97	1.93	2.58	3.29	3.64

Table 8 shows that the intermediate precision of all dyes tested at 10 mg/kg level is below 5 % RSD which is within the acceptable limit.

4.2.3 Reproducibility (Inter Laboratory Comparison)

Reproducibility represents the precision obtained between different laboratories. The objective is to verify that the method will provide the same results in different laboratories. The reproducibility of an analytical method is determined by analyzing aliquots from homogeneous lots in different laboratories with different analysts and by using operational and environmental conditions that may differ from, but are still within, specified parameters of the method (interlaboratory tests). Reproducibility, expected to give the smallest variation in results, is a measure of the variability in results between laboratories. To estimate the reproducibility of the test method, two dyes samples that contain Cr(VI) were sent to seven accredited testing laboratories along with the in-house developed test method. The round-robin test results were collected and the robust Z score of each laboratory was calculated. The ILC results are tabulated in Table 9.

Table 9 data shows that the robust Z score value of all the eight laboratories is within the acceptable limit of below ± 2.0.

Table 9 Reproducibility of Cr(VI) in water soluble dye

	Dye		Dye	
	Reactive red		**Reactive blue**	
Lab code	**Cr(VI) (mg/kg)**	**Z Score**	**Cr(VI) (mg/kg)**	**Z score**
01	9.69	-0.77	9.38	-1.32
02	9.5	-1.48	9.8	0.10
03	9.79	-0.39	10.2	1.46
04	10.31	1.56	9.92	0.51
05	10.07	0.66	10	0.78

	Dye		Dye	
	Reactive red		Reactive blue	
Lab code	Cr(VI) (mg/kg)	Z Score	Cr(VI) (mg/kg)	Z score
06	10.04	0.54	9.6	-0.58
07	9.64	-0.96	9.49	-0.95
07	9.74	-0.58	9.74	-0.10
07	10.1	0.77	9.4	-1.26
08	10	0.39	9.9	0.44
Median (Q2)	9.89		9.77	
Lower Quartile (Q1)	9.70		9.52	
Upper Quartile (Q3)	10.06		9.92	
IQR	0.36		0.40	
IQR X 0.7413	0.27		0.29	

4.3 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample. Linearity may be directly demonstrated on the analyte, or spiked samples using at least five concentrations over the whole working range. Evaluation of the analyte signal as a function of the concentration, appropriate statistical calculations are recommended, such as linear regression.

To plot a calibration graph of Cr(VI) standard solution, the first 1000 mg/kg of stock solution was prepared by exactly weighing 2.8292 grams of dried $K_2Cr_2O_7$ in 1000 ml distilled water. From this 1000 ppm solution, an intermediate solution of 10 mg/kg was prepared. From 10 mg/kg of standard solution a series of calibration solutions were prepared by pipetting out 0.5ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, and 6.0 ml into seven 25 ml volumetric flasks. These flasks were three quarters filled with distilled water and 0.5 ml H_3PO_4(2.2.2) and 0.5 ml DPC (2.2.1) solutions were added and makeup to the mark. The concentration of Cr(VI) in these solutions will be 0.2,0.4,0.8,1.2,1.6, 2.0 and 2.4 mg/kg respectively. The optical density of these colored solutions was measured at 540 nm wave length against a blank reading. A seven-point calibration graph was plotted, absorption verses concentration. Figure 1 represents a linear regression graph of Cr(VI) when plotted in the range of 0.2 to 2.4 mg/kg at 540 nm wave length. The regression coefficient (r2) of the calibration graph was 0.9990 which indicates Cr(VI) solution gives good linearity over a range of 0.2-2.4 mg/kg.

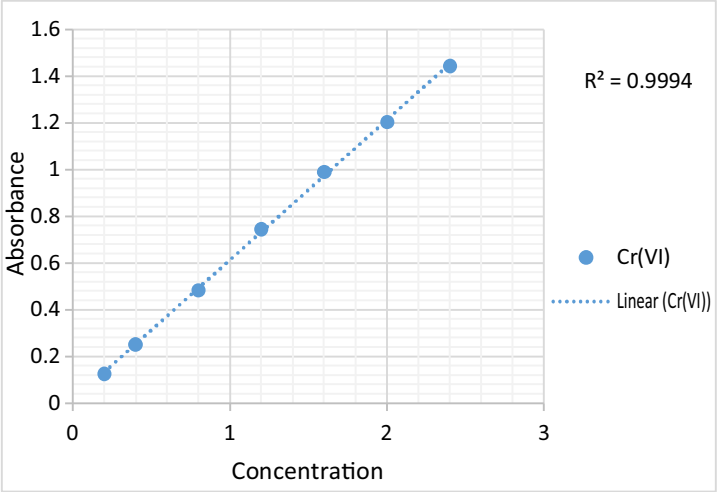

Figure1 Calibration graph of Cr(VI)

4.4 Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. The range of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written. The range is normally expressed in the same units as the test results. From figure1 it can be seen that Cr(VI) gives good linearity in the working range of 0.2 -2.4 mg/kg.

4.5 Limit of Detection (LOD)

LOD can be defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. This is also known as the instrument detection limit. The instrument detection limit can be calculated based on a standard deviation of the response based on the standard deviation of the blank or standard deviation of the response based on the slope of the calibration curve. A specific calibration graph (Figure.1) is studied using samples containing analyte in the range of limit of detection. The residual standard deviation of the y-intercepts of regression lines may be used as the standard deviation.

4.6 Limit of Quantification (LOQ)

LOQ can be defined as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. This is also known as the method detection limit.

Table 10 shows IDL and MDL of Cr(VI) by using a Spectrophotometer calculated based on a calibration graph. This indicates that a spectrophotometer can detect down up to 0.07mg/kg Cr(VI). Hence, by considering the weight of dye and final volume of extract, Cr(VI) in dyes can be quantified down up to 3.0 mg/kg.

Table 10 LOD of Cr(VI)

Calibration standards	Concentration (mg/l) X axis	Absorbance Y axis
Std-1	0.2	0.127
Std-2	0.4	0.254
Std-3	0.8	0.254
Std-4	1.2	0.746
Std-5	1.6	0.993
Std-6	2.0	1.204
Std-7	2.4	1.445
Regression coefficient (R^2)	0.9994	
The slope of the curve (M)	0.60008	
Std error (STE)	0.012756	
LOD=(STE/M) *3.3	0.07015	

Table 11 Measurement Uncertainty of Cr(VI) in different dyes

Dyes	Reactive Red 218	Yellow HE 6G	Turquiose Blue HGN	Navy Blue RX	Reactive Black 5A
Measurement uncertainty (MU) mg/kg	At 9.49 ppm ±0.16 ppm	At 9.63ppm ±0.13ppm	At 9.35ppm ± 0.26ppm	At 9.29ppm ±0.29ppm	At 9.40ppm ±0.30ppm

4.7 Measurement Uncertainty

Uncertainty is a parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand. When estimating the uncertainty of measurement, all uncertainty compounds which are important in the given situation shall be taken into account using appropriate methods of analysis. Measurement uncertainty, in general, has many components. Some of these components may be evaluated from the statistical distribution of the results of a series of measurements and can be characterized by experimental standard deviation. Other components, which also can be characterized by standard deviations, are evaluated from assumed probability distributions based on experience or other information. The calculated measurement uncertainty (MU) of Cr(VI) in five dyes at 10 ppm level is given in Table 11. (Since the measurement uncertainty calculation involves a lot of statistical parameters, each step has not been elaborated here. The only final calculated value mentioned). The reactive red dye which contain10 ppm Cr(VI), measured concentration is 9.49 ppm and can be reported with an accuracy ranging from 9.33 to 9.65 ppm based on the measurement uncertainty. Similarly Reactive black at 9.4 ppm level, accuracy will be in the range of 9.1 to 9.7 ppm.

5.0 Conclusions

Chemical speciation of Cr(VI) in water soluble dyes is not possible either by microwave digestion or by conventional digestion method as Cr (VI) reacts with all acids and loses at high temperature. The potential of activated carbon for the adsorption of dye molecule and recovery of Cr(VI) in an alkaline medium has been explored in this test method. This test method was found to be one of the best economical methods for the determination of Cr(VI) in water soluble dyes. By this method Cr(VI) in water soluble dyes were found to have good repeatability and reproducibility. The round robin test conducted with seven laboratory results agrees that the method has good reproducibility. By this method Cr(VI) in water soluble dyes can be quantified down up to 3.0mg/kg level.

Hence, this in-house developed and validated test method can be used to test Cr(VI) in all types of water soluble dyes.

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