

CHAPTER 1

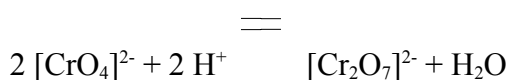
INTRODUCTION

1.1 Hexavalent Chromium (Cr⁶⁺)

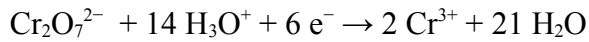
There is increasing awareness of the presence of hexavalent chromium in the environment and the effects on human health. Chromium is a grey, hard metal and is one of the most widely distributed heavy metal in the Earth crust. It is found throughout the environment in three major oxidation states which are Cr (0), Cr (III) and Cr (VI). The most stable form is Cr (III), occurs naturally, while Cr (VI) and Cr (0) are generally introduced into the environment by various industrial processes and anthropogenic activities.

Chromium does not occur freely in nature. The main chromium mineral is chromite (FeOCr₂O₃) in which it is the only ore containing a significant amount of chromium. However, the ore has not been found in the pure form; its highest grade contains about 55% chromic oxide (I). As was mentioned earlier, chromium compounds can be found in waters at trace amounts. The element and its compounds can be discharged in surface water through various industrial processes. It is applied for example for metal surface refinery and in alloys. Stainless steel consists of 12-15% chromium.

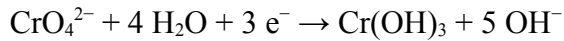
Chromium(VI) compounds are strong oxidants at low or neutral pH. Most important are chromate anion (CrO₄²⁻) and dichromate (Cr₂O₇²⁻) anions, which exist in equilibrium:



Both the chromate and dichromate anions are strong oxidizing reagents at low pH:



They are, however, only moderately oxidizing at high pH:



Chromate, CrO_4^{2-} , and dichromate, $\text{Cr}_2\text{O}_7^{2-}$, are oxidising agents often used in laboratories to clean organic material from the surfaces of glassware. This may lead to the assumption that when Cr(VI) compounds come into contact with the organic matter of living beings, it reacts in a harmful way. (Skovbjerg, 2005).

Hexavalent chromium is one of the toxic heavy metals with high mobility in soil and ground water which can produce harmful effects on organisms including humans. Hexavalent chromium [Cr (VI)] compounds are used in a wide variety of commercial process such as chromite ore processing, electroplating and leather-tanning processes, among others (Chuan and Liu, 1996; Lawson, 1997). The unregulated disposal of chromium containing effluents has led to the contamination of soil, aquatic sediments and surface and groundwater environments. The metal industry mainly discharged trivalent chromium, Cr^{3+} .

Hexavalent chromium can also be produced by the "hot work" process such as welding on stainless steel or melting chromium metal. In high temperature process, the chromium is not originally hexavalent, but the chromium will then oxidized to

hexavalent chromium in which hexavalent chromium compounds are genotoxic carcinogen.

Chromium is released to air and water during manufacturing and to water and soil when chromium-containing products are used or deposited as waste (Skovbjerg, 2005). It is often released into the environment following the disposal of chromium-containing materials or as a by-product of the processes that use this metal.

Wastewater or industrial effluent usually contains about 5 mg/L (ppm) of chromium. Chromium may be applied as a catalyzer, in wood impregnation, in audio and video production and in lasers. Chromite is the starting product for inflammable material and chemical production. Chromium may be present in domestic waste from various synthetic materials. Through waste incineration it may spread to the environment when protection is insufficient.

1.2 Environmental effects and health effects

Cr(VI) has high environmental mobility and can originate from anthropogenic and natural sources (Zhitkovich, 2011). Cr(VI) is a known carcinogenic contaminant sometimes leaked from industry or leached from fly ash to soil and groundwater. It is soluble and toxic in low concentrations (Skovbjerg, 2005). Cr(III) is considered nontoxic, even in relatively high concentrations and is an essential trace element required for the maintenance of glucose, lipid and protein metabolism. To be considered toxic, Cr (III) needs to be present in much higher concentrations than does Cr (VI).

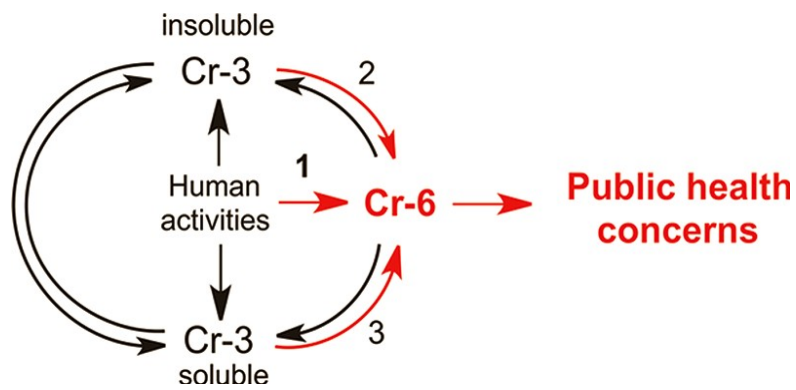


Figure 1 : Origin and interconversion of different forms of Cr in water. Route 1 is the most important overall source of Cr(VI), whereas route 2 describes the most important natural process of Cr(VI) formation. (Zhitkovich, 2011).

Chromium (VI) compounds are stable under aerobic conditions, but are reduced to chromium (III) compounds under anaerobic conditions. The reverse process is another possibility in an oxidizing environment. Chromium (VI) compounds are divided up in water hazard class 3 and are considered very toxic. Chromium (VI) compounds are toxic at low concentrations for both plants and animals. The mechanism of toxicity is pH dependent.

Hexavalent chromium is known for its negative health and environmental impact and its extreme toxicity and mutagenic. It causes allergic and asthmatic reactions, is carcinogenic and is 1000 times as toxic as trivalent chromium. On inhalation, it can cause nasal discomfort, such as nosebleed and ulcers. Ingestion of larger amounts may lead to stomach ulcers, kidney and liver damage and even death. Cr (VI) present in leather can cause skin irritation and allergy. However it is cancer that poses the biggest threat of long-time exposure to Cr (VI), such as might happen if one drinks contaminated water.

The human body contains approximately 0.03 ppm of chromium. Daily intake strongly depends upon feed levels and is usually approximately 15-200 µg, but may be as high as 1 mg. Chromium uptake is 0.5-1%, in other words very small. Toxic effects may be passed on to children through the placenta. The lethal dose is approximately 1-2 g. Most countries apply a legal limit of 50 ppb chromium in drinking water.

Several analytical techniques exist for determining trace levels of chromium, most of which are based on Atomic Absorption Spectrometry (AAS) and Inductively Couple Plasma Mass Spectrometry (ICP-MS) or Atomic Emission Spectrometry (AES). All these methods although quite sensitive require expensive instrumentation and skilled manpower. Other reported methods include titrimetric, High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Flow Injection Analyzer (FIA).

1.3 Release of Chromium to the Environment

High concentration of chromium may cause serious illness; thus most countries operate with limits for drinking water and wastewater. The guideline for total chromium in drinking water given by the World Health Organization (WHO) is 0.05 mg/L. Although hexavalent chromium is much more toxic than Cr in its other valence states, WHO provides no specific limit value, because Cr (VI), as a separate species, apparently is too difficult to measure (WHO, 2004). However, essentially all Cr present in aqueous form is Cr (VI) because of the low solubility of Cr (III) compounds.

In Malaysia, specific legislative regulations Environmental Quality Act 1974 (Industrial Effluent) Regulations 2009 exist for the treatment and quality of water. Wastewater is

any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. In the most common usage, it refers to the industrial/ or municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources.

The limit of Hexavalent Chromium in effluent of standard A is 0.05 mg/L and standard B is 0.05 mg/L. Standard A is applicable to effluent that is discharge into any inland water within catchment areas in the 3rd Schedules Environmental Quality Act 1974. Standard B is applicable to any other inland water or Malaysian waters.

In Malaysia, the various marine water quality monitoring programs represent and essential element in the overall management of the marine environment in the country. The limit of the Hexavalent Chromium in marine water is listed in the Table 1.

Table 1: Marine Water Quality Criteria and Standards (MWQCS) for Malaysia

Parameter	Class 1	Class 2	Class 3	Class E
Uses	Preservation, Marine Protected areas, Marine Parks	Marine Life, Fisheries, Coral Reefs, Recreational and Mariculture	Ports, Oil & Gas Fields	Mangroves Estuarine & River-mouth Water
Chromium (VI) ($\mu\text{g/L}$)	5	10	48	10

The Hexavalent Chromium (Cr^{6+}) by Colorimetric Method in waste water is determined by referring to American Public Health Association, Standard Method 3500 Cr B Colorimetric Method, 21 st Edition.

1.4 Discrete Analyzer



Figure 2: Smart Chem 200, Discrete Analyzer

The hexavalent chromium concentration can be determined by Colorimetric Method. In this research, the Discrete Analyzer is used to determine hexavalent chromium in seawater, river water and industrial effluent.

Discrete Analyzer is a high resolution, dual beam digital detector. It is fully automated, direct read photometry utilizing a true discrete, direct read measurement system and reusable. Discrete Analyzer employs a robust robotic sampling arm working in conjunction with a stepper motor-driven syringe that is responsible for aspirating, dispensing and mixing accurate and precise quantities of sample and reagent in miniaturized test tubes called cuvettes. Each reaction takes place in its own optical cuvette.

The sample and reagents are incubated in the reaction wells “cuvettes” for a preprogrammed time. The sample is run at a temperature of 37 °C that will increase the

rate reaction and the time required to reach a stable colorimetric endpoint absorbance. A single aliquot is then transferred into a glass optical cuvette. The absorbance is then read on the stationary reactant to ensure the best possible signal to noise ratio.

The reaction times are user programmable from seconds to minutes. The standard methods supplied have optimized reaction times which ensure the reaction is brought to completion. Once the absorbance is read, the cuvette is thoroughly cleaned to eliminate any carry over or cross contamination. (SmartChem 200 User Manual, 2005)

The advantages of the direct read discrete analysis are:

- (i) Each reaction take place in its own optical cuvette, thus can eliminates carryover or cross contamination.
- (ii) Pre and Post dilution of samples and calibration standards.
- (iii) No excessive reagent consumption.
- (iv) True sample blanking performed directly in each cuvette.

1.4.1 Components of Discrete Analyzer

There are four important components in the Discrete Analyzer which are the sampling system, reaction plate or cuvette, cuvette wash station and detector.

(a) Sampling System and Dilutor

One mechanic arm and has liquid pre-heater provides all sampling operation with capacitive liquid level sensor 2 mm immersion depth make probe washing easy, reagent pre-warming at 37 °C and automatic probe washing after every liquid contact to prevents cross contamination and carryover. There are three stages

process for probe washing which are clean the inside of the probe, clean the washing well, then clean the outside of the probe. The dilutors can carry out up to 100 times dilution for standards and samples. The sample volume and reagent volume capacity are within 3.0 μ L to 300 μ L for every sampling. This computer controlled micro pipettor (probe) can precisely dispensing for all liquids and is an integral sensor for low level alerts.

(b) Reaction Plate or Cuvette

There are a total of 60 semi-disposable cuvettes tray and it is automatic cuvette washing. This cuvette is 1 cm pathlength and can be filled up to 450 μ L of sample volume. The cuvette can be reusable for at least 2 or 3 months. The incubation temperature is 37°C.

(c) Cuvette Wash Station

This wash station is the place where the washing, rinsing and drying of cuvettes are performed. The Cuvette Quality Assurance System has been installed in this wash station in order to give high performance of washing, rinsing and drying. It also separates the hazardous waste. All cuvettes are tested for optically purity before used. If SmartChem detects a cuvette that fails outside the above range, for any filter wavelength, it will be "Flagged" and not used in any further analysis. Then, Smartchem bypasses " bad " cuvettes. A maximum 10 "bad" cuvettes can exists before SmartChem will alert the user to replace cuvettes.

(d) Detector - Optical Filter

The detector of Discrete Analyzer is a dual beam photometer and high resolution with 22-bit digital detector. Its absorbance ranging from 0.001 to 2.500 Abs and the resolution is 0.0001 Abs. One analyzer uses a spectral isolation optical system,

in which a cuvette assembly containing nine optical filters , 420 - 480 - 510 - 520 - 550 - 570 - 630 - 660 and 880 nm , and 1 dark position is computer controlled. Halogen lamp 6V/10W is used as light source. Each cuvette is read at nine wavelengths and the computer interprets only the specific wavelength for a requested test where the end point of a reaction is monitored at a single wavelength specific to the reaction chemistry used.

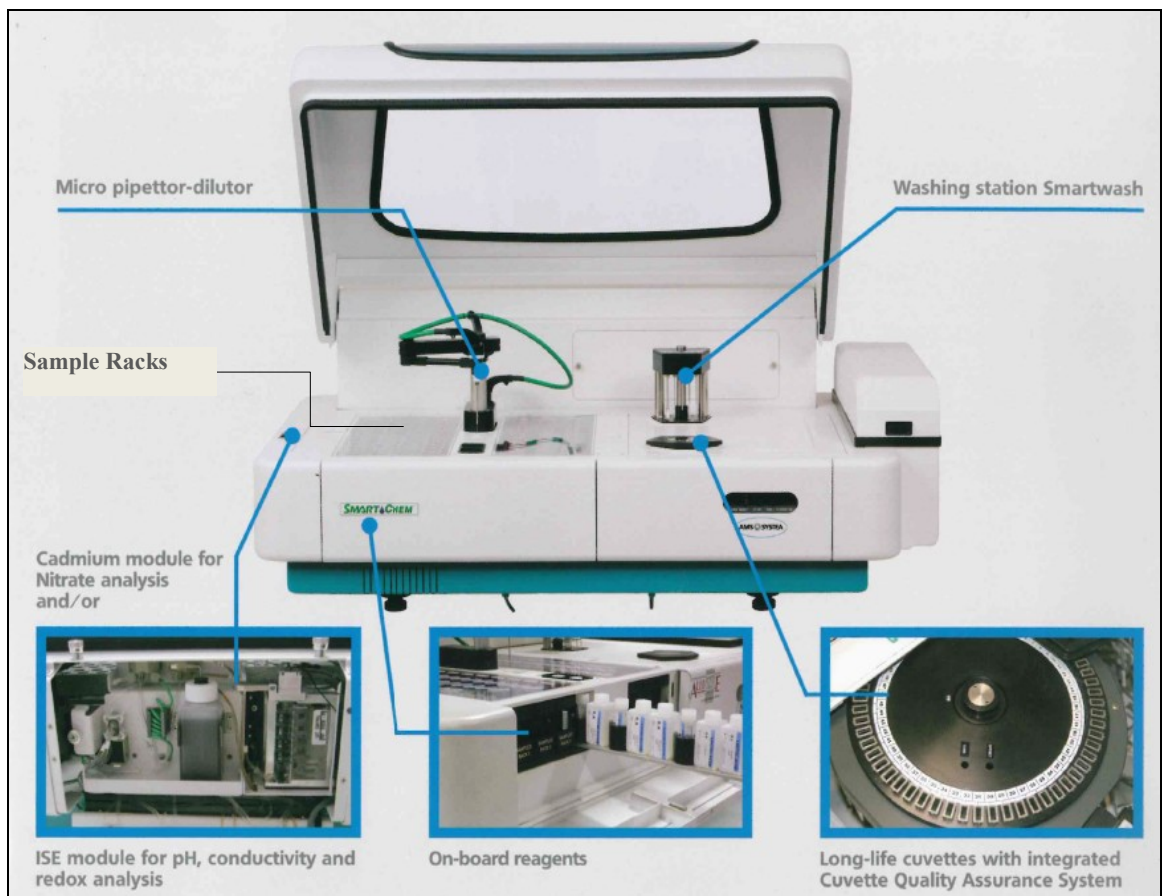


Figure 3: Components of Discrete Analyzer

1.4.2 Operation of the Discrete Analyzer

The sample tray is loaded with the specimens to be analyzed. A probe aspirates a precisely measured aliquot of sample and discharges it into the reaction vessel; a measured volume of diluent then rinses the probe. Reagents are dispensed at precisely measured volumes into the reaction vessel.

After the solution is mixed (and incubated, if necessary), it is either passed through a colorimeter, which measures its absorbance while it is still in its reaction vessel or aspirated into a flow cell, where its absorbance is measured by a flow-through colorimeter. The analyzer then calculates the analyte's chemical concentrations. The sample is aspirated from the stoppered tube and added to each cuvette, where it is mixed with reagent.

With these systems, light from a single source, halogen lamp, is broken into nine beams, 420 - 480 - 510 - 520 - 550 - 570 - 630 - 660 and 880 nm. Each beam is transmitted through light guides to one of nine photometer stations, where it is directed at the moving cuvette track. Readings are taken at the appropriate wavelengths at each station. The light that passes through the cuvette is measured by photomultiplier tubes; the measurements are sent to a microprocessor for analysis. If a sample's analyte concentration exceeds the procedure's range of linearity, the system notifies the operator, who can retrieve the sample and rerun it in the dilute mode.

1.5 Sampling of the Hexavalent Chromium sample

Sampling is performed in accordance with the instruction in the Standard Methods for the Examination of Water and Waste Water 21 st Edition. The representative samples were collected in a clean plastic bottle and the sample must be stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and analyze within 24 hours after sampling. If the sample cannot be analyzed within 24 hours, the sample must be preserved with 1M NaOH to pH 9 and stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and the sample can be held for up to 30 days. (American Public Health Association, 2005)

1.6 Objective of the study

This method is validated by referring to the APHA 3500 B- Cr Colorimetric Method, 21st Edition. The current colorimetric method of determination of Hexavalent Chromium in wastewater is carried out by using UV-VIS Spectroscopy. In order to analyze the low concentration up to ppb ($\mu\text{g/L}$) of Hexavalent Chromium in seawater, this method is developed by using Discrete Analyzer.

The objectives to carry out this study include :

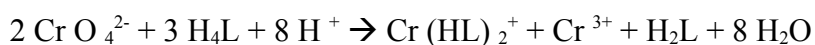
- i. Identification and development of an analytical method for the determination of the hexavalent chromium in seawater by colorimetric method using Discrete Analyzer up to ppb level. Meanwhile can analyze for the River Water and Industrial Effluent as well.
- ii. To reduce the hazardous waste as small volume of samples, standards and reagents were used in the analysis by using Discrete Analyzer.

1.7 Principle of the method

This procedure measures only hexavalent chromium. The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution where the red-violet color of unknown composition will be produced. The absorbance is measured at 550 nm.

1.8 Reaction of Cr⁶⁺ and Diphenylcarbazide

Chrome (VI) or hexavalent chromium reacts with 1,5-diphenylcarbazide in acid solution to form a red-purple chrome at pH 2 ± 0.5 . Incubation temperature is 37°C. A proper pH control is necessary for this reaction to occur. If the pH is too high, it will slow down the reaction to be ineffective. If the pH is too low, it will produce the sulfur dioxide gas at pH < 2.



H₄L : 1,5- Diphenylcarbazide

H₂L : Diphenylcarbazone

Figure 4 : The chemical reaction between Chrome (VI) and diphenylcarbazide in acidic solution

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Aspects of Hexavalent Chromium Determination

Different toxicity of the chromium species will have different chemical characteristics. The measurement of the total chromium cannot be used to determine the actual environmental impact. This is because the Cr(III) in its biologically active form will facilitate interaction of insulin with its receptor site, influencing glucose, protein and lipid metabolism, so Cr(III) is essential for animals and human beings. However, for the Cr(IV) is highly toxic and carcinogenic in which it will cause gene mutation. Thus, chemical speciation of chromium in environmental samples is necessary to accurately assess for its pollution levels toward environment and human. Recently there are sensitive analytical methods for the determination and speciation of chemical forms of toxicity of this hexavalent chromium metal ions in environmental samples.

Trivalent chromium has lower human toxicity due to its low solubility and less absorbed by organic molecules (Lokothwayo, 2007). The Cr(VI) form is a strong oxidizer and it is very toxic. Most publications pertaining to chromium speciation in environmental samples are in aqueous environments which the chromium(III) and Chromium(VI) is commonly exists in the environment.

There are many researches that have been focused on the separation and detection of dissolved chromium species in aqueous mixtures or in relatively clean liquid environmental samples. Organics do not reduce Cr(VI) in alkaline solution. Thus, the sodium

hydroxide has been used for preservation for Hexavalent Chromium. Several methods have been studied and used for chromium determination in environmental samples based on coprecipitation, ion exchange and solvent extraction, followed by spectrometric, chromatographic and electrochemical detection.

Adsorptive stripping voltammetry is the most sensitive, convenient and cost effective analytical methods for determination of Cr (VI) in aqueous samples. Moreover, High-performance liquid chromatographic (HPLC) method with diode array detection based on the chelating agent ammonium pyrrolidine dithiocarbamate has been developed for the analysis of chromium species (Cr^{3+} , CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$).

As described in US Environmental Protection Agency (EPA) Method, there is another method application of hexavalent chromium was determined (as chromate) by using ion chromatography (IC). This method specifies the use of a high-capacity IonPac AS7 anion-exchange column and UV-Vis detection after post column reaction with diphenylcarbazide reagent for the analysis of chromate in drinking water as well as in groundwater and industrial effluents. This method permits a detection limit of 0.4 ppb.

The wastewater samples were analyzed for hexavalent chromium by using colorimetric method with diphenylcarbazide in acid solution as described in American Protection and Health Association (APHA) method. A red-violet colour of unknown composition is produced which is measured at 540 nm. However, the determination of chromium by (EU) spectrophotometric method based on oxidation of organic compounds and formation of ion associates has the disadvantage of high blank value which it may suffers severe interference from Fe(III), Mo(VI), Cu(II) and Hg(II). The reaction with diphenylcarbazide is nearly specific for chromium. Hexavalent molybdenum and

mercury salts will react to form colour with the reagent but the intensities are much lower than that for chromium at specified pH. Vanadium will interfere strongly but if the concentration is up to 10 times that of chromium will not cause any interference. Iron in concentrations greater than 1 mg/L may produce a yellow colour but the ferric ion (Fe^{3+}) colour is not strong and no difficulty is encountered normally.

2.2 Water Pollution

Water pollution has been a serious environmental issue to mankind since last few decades. The common polluting anthropogenic sources are domestic sewage, mine drainage and industrial effluents. Natural weathering of rocks, which contributes a significant content of toxic elements to surface water such as ground water, river water and sea water acts as a background to the pollution impact.

CHAPTER 3

METHODOLOGY

3.1 Hexavalent Chromium

3.1.1 List of Standard

- a) 500 mg/L Stock Chromium Solution
- b) 5 mg/L Standard Solution
- c) 0.05 mg/L Standard Solution

3.1.2 List of Chemicals/ Solvent

- a) Acetone
- b) Sulphuric Acid
- c) Nitric Acid
- d) Hydrochloric Acid
- e) Probe Rinse Solution, Alliance
- f) Cuvette Cleansing Solution, Alliance
- g) Diluent/ Reagent Water (Deionized Water)

3.1.3 Apparatus

- a) Discrete Analyzer
- b) Beakers
- c) Sample Cups
- d) Volumetric Flasks
- e) Micropipettes and Tips

3.2 Procedures

3.2.1 Preparation of Reagents

a) Acid Reagent : 0.2 N Sulphuric Acid

0.2 N Sulphuric acid is prepared by adding 20 ml of deionized water into 100 ml of volumetric flask and then dissolved 0.56ml concentrated sulphuric acid into it. Then, the solution is make up to volume with deionized water.

b) Diphenylcarbazide Reagent (DIPH)

0.250 g 1,5-diphenylcarbazide $C_{13}H_{14}N_4O$ is weighed and then dissolved into 25 ml Acetone and mixed until it is completely dissolve. The solution is then make up with deionized water to a final volume of 50 ml volumetric flask. This reagent is then stored in an amber glass bottle and prepare weekly. Discard if the solution becomes discolored.

c) Reagent Water

Deionized water, free of the analyte of interest is used as reagent water or diluent.

3.2.2 Preparation of Probe Rinse Solution and Cleaning Solution

a) Cuvette Cleaning Solution

In a one litre volumetric flask containing about 500 ml of deionized water, add 50 ml of cleaning solution and dilute to one litre with deionized water and mix thoroughly.

b) Probe Rinse Solution

In a one litre volumetric flask containing about 500 ml of deionized water add 0.5 ml of rinse probe solution and dilute to one litre with deionized water and mix thoroughly.

3.2.3 Preparation of Standard Solution

a) Stock Chromium Solution, 500 mg/L

141.4 mg of $K_2Cr_2O_7$ is weighed and dissolved in deionized water and dilute to 100 mL. Do not keep the solution more than 6 months. (1.00 mL=500 μ g Cr)

b) Standard Chromium Solution, 5 mg/L

1 ml of stock standard solution 500 mg/L is diluted to 100 ml with deionized water and mix thoroughly. Prepare freshly.

c) Quality Control Standard Solution, 0.05 mg/L

1 ml standard solution 5 mg/L is diluted to 100 ml with deionized water and mix thoroughly. Prepare freshly.

3.2.4 Preparation of Laboratory Ware

Soak all glassware and laboratory ware in a mixture of nitric acid, hydrochloric acid and deionized water with a ratio of 1 : 2 : 9 overnight. Rinse with deionized water prior to use for analysis.

3.2.5 Preparation of Sample

Turbid samples should be filtered prior to analysis to prevent interference from turbidity. Suspended particles should be removed by filtration.

3.2.6 Procedure for Loading of sample into Discrete Analyzer

- a) Fill the sample cups with sample, standard, blank and QC.
- b) Triple rinse with blank, standard, sample or QC respectively prior to pour the sample in the sample cups.
- c) Check the probe rinse, deionized water and cleaning solution bottles is reach at the appropriate level as mark at the bottles and fill as necessary prior to run for sample.
- d) Place the sample into sample rack.
- e) The diluent bottle for this method is filled with the deionized water.
- f) Fill a clean reagent bottle with the Acid Reagent "0.2 N Sulphuric Acid" and then fill a second reagent bottle with the Color Reagent " Diphenylcarbazide".

3.3 Performance Characteristic

A series of spike samples is prepared for the Performance Characteristic of determination of Hexavalent Chromium in Seawater, River Water and Industrial Effluent by diluting from intermediate standard solution 5 mg/L to concentration 0.005 mg/L, 0.05 mg/L and 0.25 mg/L. This intermediate standard stock 5 mg/L solution is prepared from 500 mg/L stock standard solution and the diluent is deionized water.

A 0.25 mg/L of the intermediate standard is prepared by spiked with 5ml from 5 mg/L of standard solution and diluted to 100 ml by deionized water. These series of the spike samples are prepared in 100 ml volumetric flask and diluted to the volume by the matrices which are river water, seawater and industrial effluent repectively. The volume taken to prepared for the series of spike samples are listed in Table 2.

Concentration of Spike Samples (mg/L)	Taken from the standard (mg/L)	Volume taken (ml)	Final Volume (ml)
5	500	1	100
0.25	5	5	100
0.05	5	1	100
0.005	0.25	2	100

Table 2: Preparation of the series of the spike samples

3.4 Method Setup in Discrete Analyzer

3.4.1 Procedure for Parameters Method File Setup in the Discrete Analyzer by using the Smartchem Software

Type	End Point
Direction	Up
Decimals	3
Model	Linear
Optical Filter (nm)	550

Method: Cr 6+	Volume	Delay	Read	Rinse
Range: 0 - 0.25 mg/L Cr 6+	(μ L)	(second)	(second)	(μ L)
Sample Volume	250			
Reagent 1 (Acid Solution)	50	144	0	0
Reagent 2 (Diphenylcarbazide)	5	0	288	0

Table 3: Method Setup for the Parameter of Hexavalent Chromium

Note : The minimum sample and reagent volume is 3 μ L. The maximum total reaction volume is 500 μ L. The method as described here is equivalent to the Standard Methods 3500- Cr B.

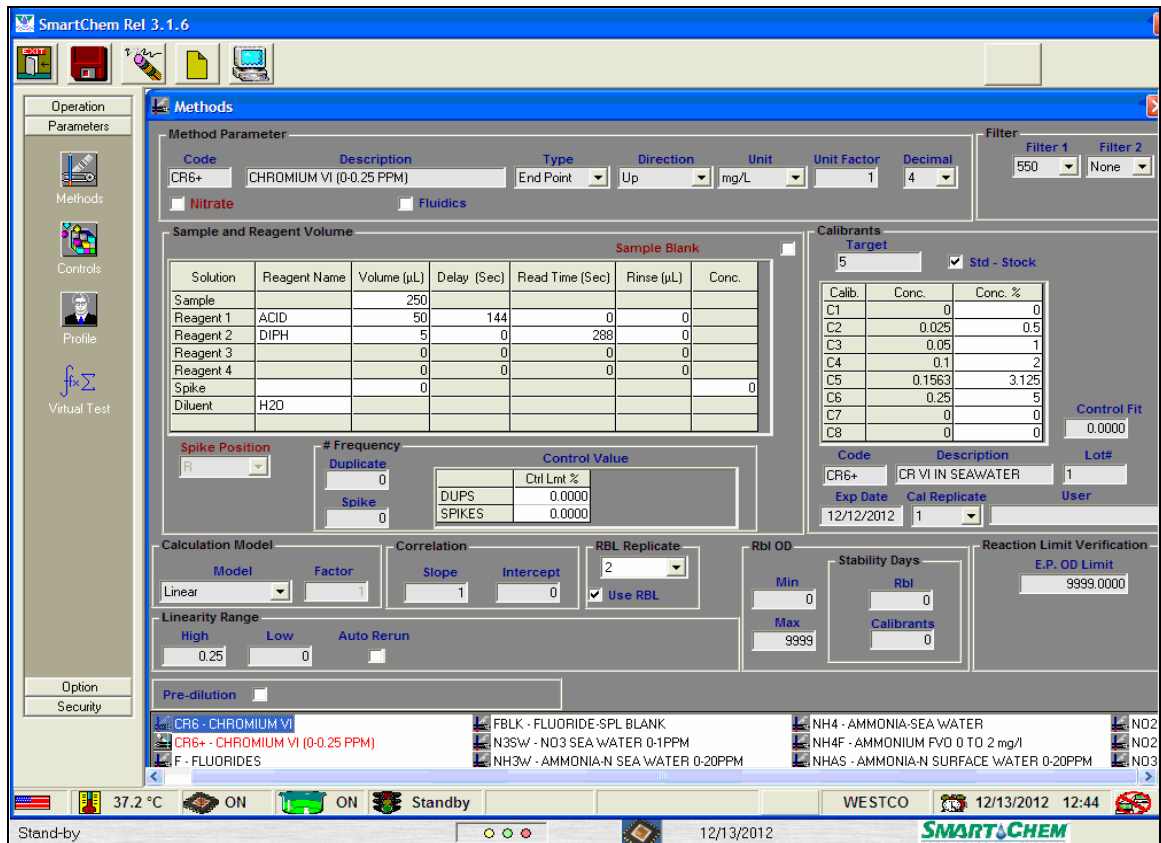


Figure 5 : Method Setup for the Parameter of Hexavalent Chromium in Smart Chem software

3.4.2 Operation Directory to create Sample Work List (Sample Entry) and Run the sample (System Monitor)

- Clicks on the “Sample Entry” icon, the screen shown in Figure 5 will be displayed.
- Select the parameter or method name in the " Profile List" and double clicks at the chosen method.
- Enter the number of sample to be loaded into the Discrete Analyzer to create for the sequence or work list. Then, click the tick " / " icon.
- Click on the ID Sample Field, key in the sequence of the sample or sample name until all the sample information has been entered. Then, press the “Enter” key to increase the field by one.

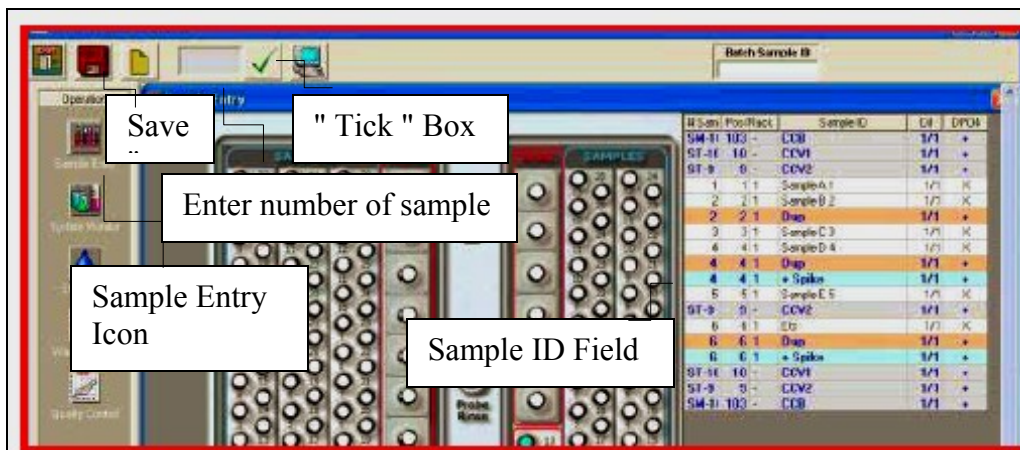


Figure 6: Sample Entry Screen

e) Click save as shown in Figure 5 to save the completed sample identification work list. Then, select “Yes” as shown in Figure 6.



Figure 7 : Saving the sample entry or work list

- f) To save the completed sample identification work list, type in a file name and select “OK.” The list will be saved and can be recalled and used in creating a sample ID work list run plan.
- g) Click on the " System Monitor" icon and select the Run Plan Work List that has been created by double click.
- h) Then, the method and work list has been loaded into the Smart Chem system and it will display the position of the sample, diluent, reagent, standard, cuvette and auto standard preparation for calibration point as shown in the Figure 7.

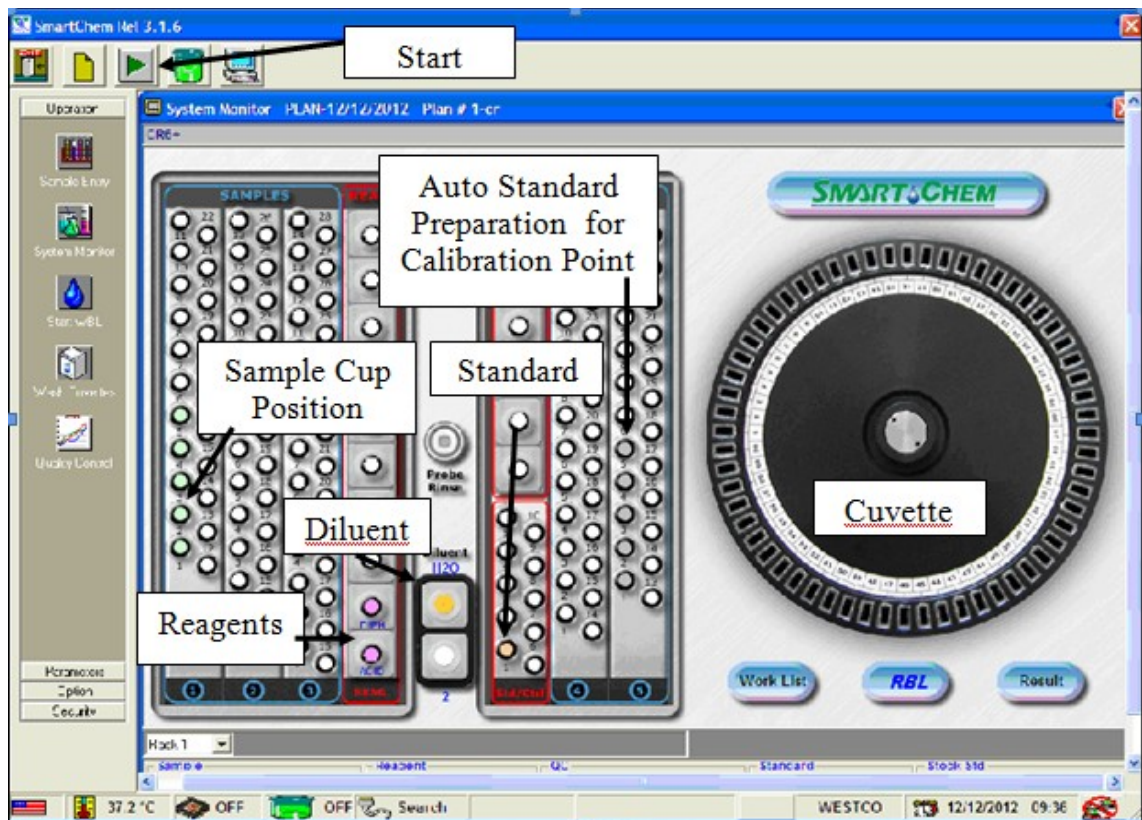


Figure 8 : Sample Work List in System Monitor

- i) After loaded all the samples, standards, diluent, reagents and empty sample cups for the auto standard preparation for the calibration point, then click the " start" button as shown in Figure 7.
- j) After press the " start" button, the Smart Chem " Play Options" box will appear. Tick on the calibration box and then click on the green button to start the run as shown in Figure 8.

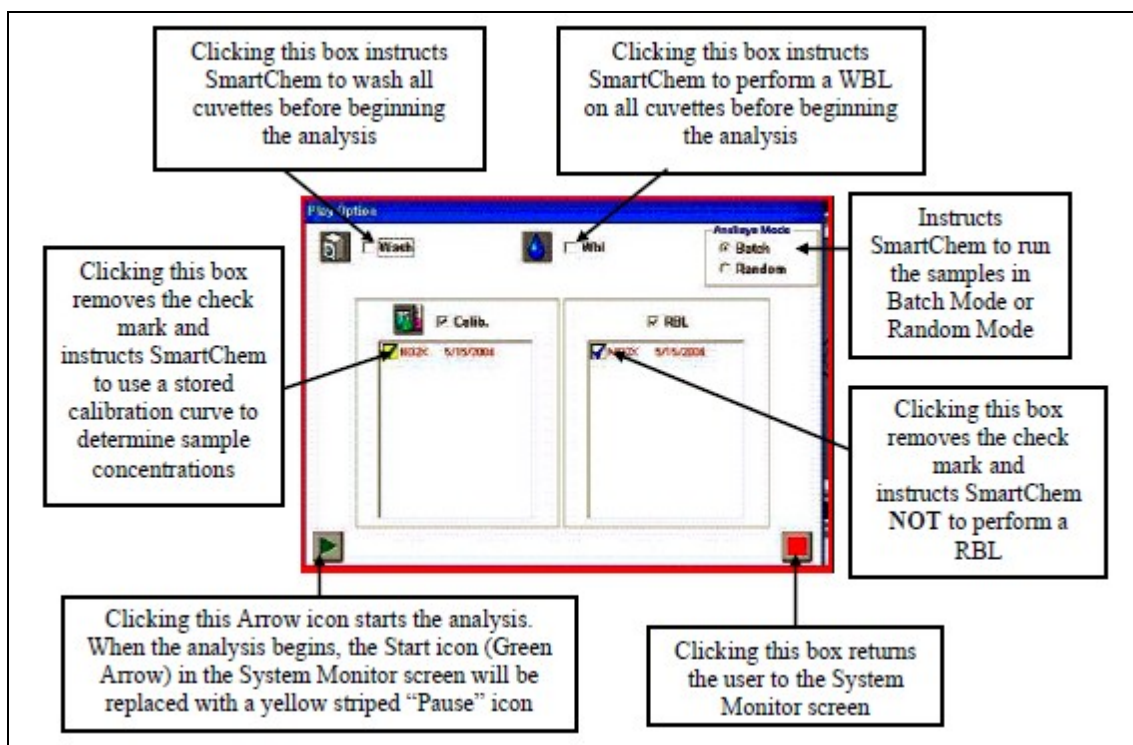


Figure 9 : Smart Chem Run Plan Option Screen

3.5 Quality Control Protocols

- The standard solution of 0.05 mg/L was used as Quality Control (QC) sample.
- A QC sample will be analyzed for every 10 samples in every batch.
- If the analysis result of the QC sample is fall outside the control limit in the Statistical Quality Control Chart, a new QC sample will be prepared and repeat for the test, if the repeated analysis result still falls beyond the limits, then a corrective action will be taken to investigate for the problem.

3.6 Calculation

The concentration of the Hexavalent Chromium is calculated as below:

$$\text{mg/L Cr}^{6+} = \text{sample value} - \text{blank value (deionized water)}$$

CHAPTER 4

RESULTS

4.1 Calibration Linearity

In this study, a 5.0 mg/L of Hexavalent Chromium standard solution is prepared and the Discrete Analyzer can be set for the automatic standard preparation for the calibration point from the standard 5.0 mg/L. The calibration curve of Absorbance (Abs) versus Concentration (mg/L) of Hexavalent Chromium is plotted. The correlation coefficient, R^2 is calculated.

4.1.1 Preparation for the calibration

- a) The 5.0 mg/l standard solution prepared by diluted in deionized water is loaded into discrete analyzer to establish a calibration curve.
- b) The discrete analyzer automatically prepares diluted standards from 0 to 0.25 mg/L in which the number point of the calibration can be selected in the Smart Chem system according to the percentage from the standard prepared as shown in Table 4 and the method set up for the calibration point for this method is shown in Figure 4 in Chapter 2.

No.	Concentration (mg/L)
1	0.0000
2	0.0250
3	0.0500
4	0.1000
5	0.1563
6	0.2500

Table 4 : Concentration of the Calibration Point for Hexavalent Chromium Method

4.1.2 Calibration Graph

Linearity can be tested by linear regression of the responses on the concentrations in an appropriate calibration set. The calibration graph of the determination of hexavalent chromium was done by plotting the absorbance versus concentration of the hexavalent chromium as shown in the Figure 9. The correlation coefficient, R^2 is 0.9998 and it shows a good linearity of the calibration graph which is almost near to 1.0000.

Concentration (mg/L)	Absorbance (Abs)
0.0000	-0.0002
0.0250	0.0124
0.0500	0.0244
0.1000	0.0483
0.1563	0.0780
0.2500	0.1224

Table 5: Data for the calibration point for the determination of hexavalent chromium

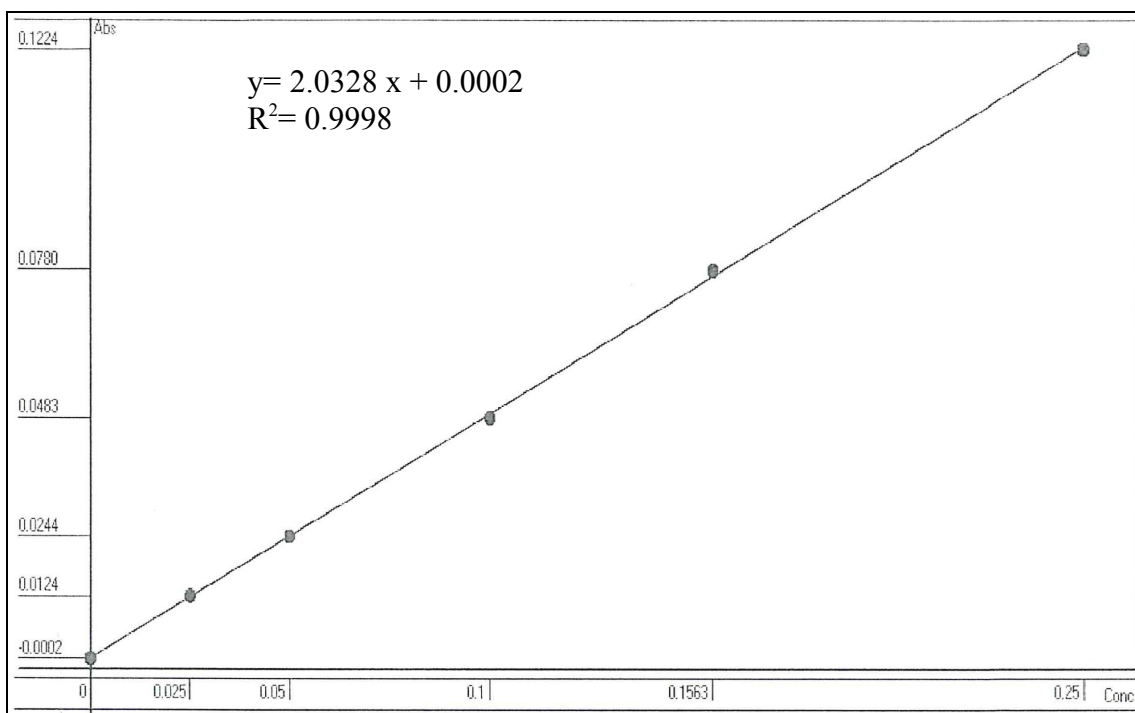


Figure 10: Calibration Curve for the determination of hexavalent chromium

4.2 Spiking level for the sample

In this method validation, there are three spiking concentration levels in seawater, river water and industrial effluent which were spiking at low, medium and high concentration level ranging from 0.005 mg/L, 0.05 mg/L and 0.25 mg/L. To obtain an accurate and precise analysis result, 7 replicate were carried out for the repeatability test and the mean value, standard deviation, relative standard deviation and recovery for the determination of hexavalent chromium in seawater, river water and industrial effluent by colorimetric method was done.

4.2.1 Data for the analysis result of the determination of hexavalent chromium in deionized water, seawater, river water and industrial effluent

Sample type / matrix	Spiking level, (mg/L)	Precision				Accuracy	
		Mean, (mg/L)	Standard Deviation, s	2s	3s	CV (%)	Recovery (%)
Deionized Water	0.0050	0.0049	0.0004	0.0009	0.0013	8.90	98.57
	0.0500	0.0502	0.0003	0.0006	0.0010	0.64	100.43
	0.2500	0.2493	0.0002	0.0005	0.0007	0.09	99.72

Table 6: Data for the analysis result of the spiked sample in deionized water

Sample type / matrix	Spiking level, (mg/L)	Precision				Accuracy	
		Mean, (mg/L)	Standard Deviation, s	2s	3s	CV (%)	Recovery (%)
Seawater	0.0050	0.0052	0.0003	0.0006	0.0008	5.28	104.54
	0.0500	0.0500	0.0006	0.0013	0.0019	1.29	100.00
	0.2500	0.2469	0.0008	0.0017	0.0025	0.34	98.75

Table 7: Data for the analysis result of the spiked sample in seawater

Sample type / matrix	Spiking level, (mg/L)	Precision				Accuracy	
		Mean, (mg/L)	Standard Deviation, s	2s	3s	CV (%)	Recovery (%)
River Water	0.0050	0.0050	0.0003	0.0006	0.0009	5.95	99.71
	0.0500	0.0492	0.0003	0.0005	0.0008	0.54	98.37
	0.2500	0.2323	0.0014	0.0027	0.0041	0.58	92.92

Table 8: Data for the analysis result of the spiked sample in river water

Sample type / matrix	Spiking level, (mg/L)	Precision				Accuracy	
		Mean, (mg/L)	Standard Deviation, s	2s	3s	CV (%)	Recovery (%)
Industrial Effluent	0.0050	0.0050	0.0002	0.0005	0.0007	4.69	99.71
	0.0500	0.0448	0.0003	0.0007	0.0010	0.75	89.57
	0.2500	0.2202	0.0018	0.0036	0.0054	0.82	88.09

Table 9 : Data for the analysis result of the spiked sample in industrial effluent

4.3 Method Detection Limit, MDL

4.3.1 MDL for Hexavalent Chromium

Sample	Analytical Result of 0.005 mg/L Cr ⁶⁺ in Deionized Water
1	0.0049
2	0.0053
3	0.0049
4	0.0041
5	0.0053
6	0.0047
7	0.0053
Mean	0.00493
s	0.0004
2s	0.0009
3s	0.0013
MDL (mg/L)	0.0010

Table 10 : Analytical Result of spiked sample 0.005 mg/L Cr⁶⁺ in Deionized Water

Note:

By referring to the Student's t table at 95 % confidence level, the MDL value is calculated as follow:

$$\text{MDL} = (t) \times (s)$$

$$t = 3.14 \times s \text{ [for 7 replicates]}$$

$$\text{MDL} : 3.14 \times s$$

Therefore, the method detection limit, MDL is determined to be 0.0010 mg/L or 1 µg/L.

4.4 Quality Control

4.4.1 Quality Control Data

20 replicates of the analysis of Quality Control data at concentration 0.5 mg/L Cr⁶⁺ for the determination of hexavalent chromium were collected and tabulated in Table 11.

Quality Control sample was analyzed after 10 samples for every batch.

No	Analytical Result /mg/L
1	0.0448
2	0.0509
3	0.0522
4	0.0451
5	0.0512
6	0.0530
7	0.0451
8	0.0496
9	0.0490
10	0.0524
11	0.0448
12	0.0510
13	0.0451
14	0.0493
15	0.0502
16	0.0488
17	0.0512
18	0.0498
19	0.0488
20	0.0514
Mean, x	0.0492
s	0.0027
2 s	0.0054
3 s	0.0081

Table 11: Quality Control data of the 20 replicates for the determination of hexavalent chromium at concentration 0.5 mg/L Cr⁶⁺

4.4.2 Quality Control Chart

Number of Data	20	% Relative Standard Deviation, RSD	5.4822
Mean	0.0492	Upper Warning Limit, +2s	0.0543
Standard Deviation, s	0.0027	Lower Warning Limit, -2s	0.0435
Variability, 2s	0.0054	Upper Control Limit, +3s	0.0570
3s	0.0081	Lower Control Limit, -3s	0.0408

Table 12: Statistical Quality Control data for the Control Limit And Warning Limit

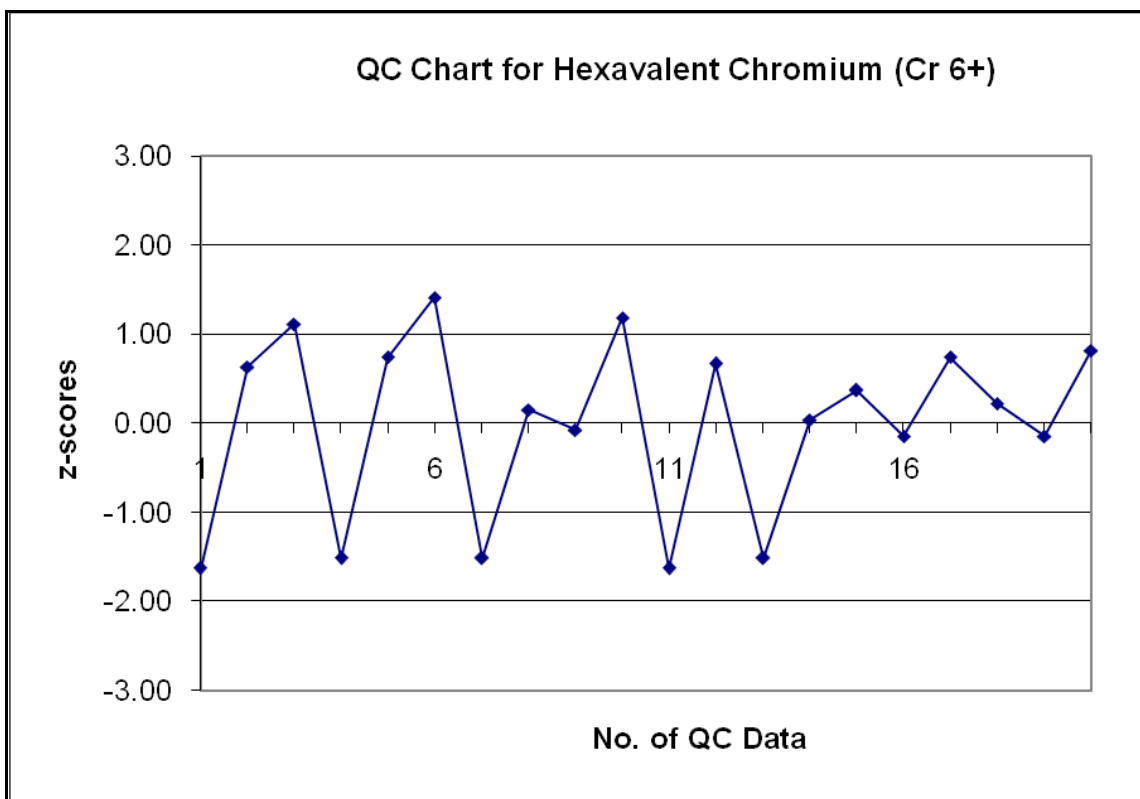


Figure 11 : QC chart for Hexavalent Chromium

4.5 Data for Measurement Uncertainty (MU)

4.5.1 MU for Cr⁶⁺

a) Precision Data

Precision Data for spiking samples of 0.005 mg/L, 0.05 mg/L and 0.25 mg/L Hexavalent Chromium in deionized water. Data was collected over a period of time. The Relative Standard Deviation (RSD) value is taken as standard uncertainty for μ (Precision).

Number of Replicates	Spiked Concentration Level (mg/L)		
	0.0050	0.0500	0.2500
1	0.0049	0.0501	0.2497
2	0.0053	0.0509	0.2491
3	0.0049	0.0501	0.2495
4	0.0041	0.0503	0.2493
5	0.0053	0.0503	0.2491
6	0.0047	0.0499	0.2493
7	0.0053	0.0501	0.2491
Mean	0.0049	0.0502	0.2493
SD	0.0004	0.0003	0.0002
RSD	0.0890	0.0064	0.0009

Table 13: Data For The Precision Of Measurement Uncertainty Of Cr⁶⁺

$$RSD_{pooled} = \sqrt{\left(\frac{(n_1 - 1) \times RSD_1^2 + (n_2 - 1) \times RSD_2^2 + \dots}{(n_1 - 1) + (n_2 - 1) + \dots} \right)} = 0.0515$$

b) Recovery

Standard uncertainty contributed to the recovery is includes method recovery (R_m) and sample recovery (R_s), so by combining both the standard uncertainty value of μ R_m and μ R_s, the Combined Uncertainty for Recovery, μ (R) can be calculated.

i. Determination of Method Recovery, R_m

The quality control level of concentration 0.05 mg/L was spiked in the Deionized Water and the 7 replicates was performed to check for the precision or repeatability of the analysis result. Thus, the uncertainty for method recovery, $\mu(R_m)$ can be calculated by using the formula as shown below and the data were tabulated as in Table 14.

Replicate	Observed Concentration, 0.05 mg/L
1	0.0501
2	0.0509
3	0.0501
4	0.0503
5	0.0503
6	0.0499
7	0.0501
Mean	0.0502
SD	0.0003
RSD	0.0064

Table 14 : Data for the Method Recovery of Method Uncertainty

$$\text{Concentration of spiked sample, mg/L} = 0.05$$

$$\text{Uncertainty due to spike solution, } \mu(C_{\text{spike}}) = 0.0029$$

$$R_m = \frac{\text{Mean concentration}}{\text{Concentration of spike solution, } C_{\text{spike}}} = \frac{0.0502}{0.0500} = 1.004$$

$$\begin{aligned} \mu R_m &= R_m \times \sqrt{\left(\frac{sd}{C_{\text{obs}}}\right)^2 \frac{1}{n} + \left(\frac{\mu C_{\text{spike}}}{C_{\text{spike}}}\right)^2} \\ \mu R_m &= 1.004 \times \sqrt{\left(\frac{0.0003}{0.0502}\right)^2 \frac{1}{7} + \left(\frac{0.0029}{0.0500}\right)^2} \\ &= 0.0583 \end{aligned}$$

At 2 Degree of freedom, $t_c = 2.262$ and t value calculated from the formula as shown.

Significant testing, t-test is calculated as below:

$$t_c = \frac{|1 - R_m|}{\mu R_m} = \frac{|1 - 1.004|}{0.0583} = 0.0686$$

The value calculated is less than 2, therefore R_m is not significant different from 1.

Correction is unnecessary.

ii. Sample Recovery

The sample were spiked at concentration level of 0.05 mg/L Cr^{6+} in various sample matrices and analyzed in 7 replicates. The mean recovery of the spiking sample in seawater, industrial effluent and river water were calculated and the data were tabulated in Table 15.

No	Repeatability at concentration 0.05 mg/L of Cr^{6+}		
	Sea Water	Industrial Effluent	River Water
1	0.0502	0.0446	0.0496
2	0.0500	0.0448	0.0493
3	0.0496	0.0453	0.0488
4	0.0498	0.0446	0.0490
5	0.0496	0.0448	0.0490
6	0.0496	0.0451	0.0493
7	0.0514	0.0443	0.0493
Mean	0.0500	0.0448	0.0492
SD	0.0006	0.0003	0.0003
RSD	0.0129	0.0075	0.0054

Table 15: Data for the Sample Recovery of the Spiking Sample In Seawater, Industrial Effluent And River Water at concentration 0.05 mg/L Cr^{6+}

SD of the mean recovery in 3 spiking matrices , $\mu R_s = 0.0028$

Therefore, combined uncertainty for recovery is calculated as below:

$$\mu (R) = \sqrt{(\mu R_m)^2 + (\mu R_s)^2}$$

iii. Calculation of Overall Measurement Uncertainty For The Method

Parameter	Description	Value, x	Standard uncertainty, $\mu (x)$	$\left(\frac{\mu x}{x}\right)$	$\left(\frac{\mu x}{x}\right)^2$
P	Precision	1	0.0515	0.0515	0.002654
Rec	Recovery	1	0.0584	0.0584	0.000014
Combined Relative Std Uncertainties					0.051652

Table 16: Data of the combined relative standard uncertainty

Combined relative standard uncertainty is calculated as below:

$$\mu (x) = \sqrt{\left(\frac{\mu Rec}{Rec}\right)^2 + \left(\frac{\mu P}{P}\right)^2}$$

Expanded Uncertainty at 95% confidence level, $K = 2$ will be

$$\mu (x) \times 2 = 0.0517 \times 2 = 0.1034$$

At any Hexavalent Chromium concentration, the uncertainty of Hexavalent Chromium in the sample will be calculated as below:

$$\mu (\text{Hexavalent Chromium concentration}) = \text{Concentration of sample} \times 0.1034.$$

Therefore, the uncertainty at 0.05 mg/L will be calculated as below:

$$\mu (x) = 0.05 \text{ mg/L} \times 0.1034 = 0.005 \text{ and it will be written as } 0.05 \pm 0.005 \text{ mg/L}$$

Hexavalent Chromium.

CHAPTER 5

DISCUSSIONS

Discrete Analyzer is a highly recommended to be used for this study due to it is highly sensitive, rapid and ease of used compared to the other type of instrument technique as it is automated method and this can reduce chemical waste and faster the time of analysis. Besides that, this analyzer is using direct photometric method in which the samples and reagents are self-mixed in a cuvette and it is sampling by using the automated probe or arm. The reaction can be reacted in a small amount of reagent and sample and it is a automated sampling method, so that no carry over and contamination of sample during the analysis. Meanwhile, the analysis result will be more precise and accurate as the sample and reagent are consistently introduce or sampling into the cuvette for reaction.

5.1 Method Validation

Method validation is a requirement of accreditation bodies, and must be supported and extended by method performance verification during routine analysis (analysis quality control). The method must be tested for sensitivity, mean recovery (as measure of trueness or bias), precision and limit of quantification (LOQ). This means that spiked recovery experiments to check the accuracy of the method should be undertaken.

5.2 Performance Characteristics

An important step in method validation of the analytical method including selectivity, calibration, trueness (bias and recovery), operating range and limit of detection. The performance such as standard deviation, variability and recovery of the study are shown in Clause 5.2.6 Summary for Performance Characteristic Data (Table 17, Table 18, Table 19 and Table 20).

5.2.1 Calibration Curves and Linearity Range

Linearity is an important property of methods used to make measurements at a range of concentrations or working range. The linearity of an analytical procedure is its ability (within a given range) to obtain analytical test results which are directly proportional to the concentration of analyte in the sample. It may be directly demonstrated on the analyte, or on spiked samples using at least five concentrations over the whole working range. In addition, a visual evaluation of the analyte signal as a function of the concentration, appropriate statistical calculations are recommended, such as a linear regression (R^2).

The parameters slope and intercept, residual sum of squares and the coefficient of correlation should be reported. A graphical presentation of the data is recommended and a good linear regression R^2 for a Linearity Calibration plot is 0.9999 which is almost near to 1.

5.2.2 Range

The range of an analytical method is the interval between the upper levels and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the by using the method as written. For assay test, it requires a minimum of specified recovery range to be within 80 to 120 percent of the analytical test concentration. This percentage recovery evaluation is to check for the method performance which the accuracy and the error of the test result can be known. In this study, the upper concentration level is at 0.25mg/L of Cr ⁶⁺ and the lower concentration level started from 0 mg/L of Cr ⁶⁺.

5.2.3 Accuracy

Accuracy is measure of how closely the result of an experiment agrees with the expected result. The difference between the obtained result and the expected result is usually divided by expected result and reported as percent relative error as calculated below:

$$\% \text{ Error} = \frac{\text{Obtained Result} - \text{Expected Result}}{\text{Expected Result}} \times 100$$

When the experimental result is within 1% of the correct results, the analytical method is highly accurate. If the percent relative error is within 1 – 5 % , it is moderately accurate, if more than 5% , it is less accuracy.

5.2.4 Precision

Precision is a degree of agreement between the average of repeated measurements of the same quantity under stated conditions. The natural random variation of measurement results, and fluctuates around the mean (random error). Precision is a measure of this variability. When a sample is analyzed for several time, the individual results are rarely the same. Instead, the results are randomly scattered. Thus, the closer the agreement between individual analyses, the more precise the results. Precision is an indication of the reproducibility of a measurement or result. The method that used for controlling precision is by using Statistical Quality Control (SQC).

5.2.5 Spike Recoveries

One of the important quality assessment tools is the recovery of a known addition, or spike, of analyte to a method blank or sample. To determine a spike recovery, the blank or sample is split into two portions, and a known amount of a standard solution of the analyte is added to one portion. The concentration of the analyte is determined for both the spiked, F , and unspiked portions, I , and the percent recovery, % R, is calculated as

$$\% R = \frac{F - I}{A} \times 100$$

where A is the concentration of the analyte added to the spiked portion.

Spike recoveries on method blanks are used to evaluate the general performance of an analytical procedure. Spike recoveries for samples are used to detect systematic errors due to the sample matrix or the stability of the sample after its collection. The typical limit for the acceptable spike recoveries of metals for the analysis of waters and

wastewater is within 80 % to 120 %. Based on the result of this study, the spiked recovery is fall within 88 % to 105 %.

5.2.6 Summary for Performance Characteristic Data

The summary of the performance characteristic data for the determination of hexavalent chromium in deionized water, seawater, river water and industrial effluent are listed in the Table 17, Table 18, Table 19 and Table 20.

Performance Characteristic		Spiking concentration of Cr ⁶⁺ (mg/L) in Deionized Water		
		0.005	0.05	0.25
Precision	Mean, $x = 7$	0.0049	0.0502	0.2493
	Standard Deviation, s	0.0004	0.0003	0.0002
	Variability, $2s$	0.0009	0.0006	0.0005
	$3s$	0.0013	0.0010	0.0007
Accuracy/ Bias	% Relative Standard Deviation, $(s/x) \times 100$	8.90	0.64	0.09
	%Error, $\frac{\text{Mean Value} - \text{Ref Value}}{\text{Mean Value}} \times 100$	1.43	0.43	0.28
	% Recovery, $\frac{\text{Mean Value}}{\text{Reference Value}} \times 100$	98.57	100.43	99.72

Table 17: Summary of the performance characteristic of the spiked sample in Deionized Water

Performance Characteristic		Spiking concentration of Cr ⁶⁺ (mg/L) in Seawater		
		0.005	0.05	0.25
Precision	Mean, $x = 7$	0.0052	0.0500	0.2469
	Standard Deviation, s	0.0003	0.0006	0.0008
	Variability, $2s$	0.0006	0.0013	0.0017
	$3s$	0.0008	0.0019	0.0025
Accuracy/ Bias	% Relative Standard Deviation, $(s/x) \times 100$	5.28	1.29	0.34
	%Error, $\frac{\text{Mean Value} - \text{Ref Value}}{\text{Mean Value}} \times 100$	4.54	0.00	1.25
	% Recovery, $\frac{\text{Mean Value}}{\text{Reference Value}} \times 100$	104.54	100.00	98.75

Table 18 : Summary of the performance characteristic of the spiked sample in Seawater

	Performance Characteristic	Spiking concentration of Cr ⁶⁺ (mg/L) in River Water		
		0.005	0.05	0.25
Precision	Mean, $x = 7$	0.0050	0.0492	0.2323
	Standard Deviation, s	0.0003	0.0003	0.0014
	Variability, $2s$	0.0006	0.0005	0.0027
	$3s$	0.0009	0.0008	0.0041
Accuracy/ Bias	% Relative Standard Deviation, $(s/x) \times 100$	5.95	0.54	0.58
	%Error, $\frac{\text{Mean Value} - \text{Ref Value}}{\text{Mean Value}} \times 100$	0.29	1.63	7.08
	% Recovery, $\frac{\text{Mean Value}}{\text{Reference Value}} \times 100$	99.71	98.37	92.92

Table 19: Summary of the performance characteristic of the spiked sample in River Water

	Performance Characteristic	Spiking concentration of Cr ⁶⁺ (mg/L) in Industrial Effluent		
		0.005	0.05	0.25
Precision	Mean, $x = 7$	0.0050	0.0448	0.2202
	Standard Deviation, s	0.0002	0.0003	0.0018
	Variability, $2s$	0.0005	0.0007	0.0036
	$3s$	0.0007	0.0010	0.0054
Accuracy/ Bias	% Relative Standard Deviation, $(s/x) \times 100$	4.69	0.75	0.82
	%Error, $\frac{\text{Mean Value} - \text{Ref Value}}{\text{Mean Value}} \times 100$	0.19	10.43	11.91
	% Recovery, $\frac{\text{Mean Value}}{\text{Reference Value}} \times 100$	99.71	89.57	88.09

Table 20: Summary of the performance characteristic of the spiked sample in Industrial Effluent

5.3 Method Detection Limits

The detection limit is a statistical statement about the smallest amount of analyte that can be determined with confidence. The International Union of Pure and Applied Chemistry (IUPAC) defines the detection limits as the smallest concentration or absolute amount of analyte that has a signal arising from a reagent blank.

The method detection limit (MDL) is defined by the EPA (USEPA, 1986) as the lowest concentration of an analyte in which it is reportable with 99% confidence that the value is greater than zero. It is determined by analyzing seven replicates of a standard solution with a concentration of hexavalent chromium near the estimated detection limit and multiplying the standard deviation (SD) by the Student's t value for degrees of freedom (n-1) of 6 and $(\alpha-1) = 0.99$ ($MDL = SD * 3.143$ for $n= 7$, at 95 % confidence level).

Meanwhile, the Lowest Concentration Minimum Reporting Limit (LCMRL) is defined as “ the lowest spiking concentration such that the probability of spike recovery in the 50% to 150% range is at least 99%”. Details of the method used to determine the LCMRL are given by USEPA (2010f). The LCMRL will be greater than the MDL.

The Minimum Reporting Level (MRL) is the minimum concentration that can be reported by a laboratory as a quantified value (USEPA, 2010b). The MDL is referenced by many older methods and data falling below the MDL were often censored or reported as “below detection limit.” USEPA is now moving towards using the LCMRL and MRL to determine if data meet quality control criteria. (Water Research Foundation, 2002). From this study, the theoretical MDL (3s) of this method is calculated as 0.001 mg/L or 1 ppb.

5.4 Statistical Quality Control (SQC) Data

A quality control procedure must involve the monitoring of data over some period of time. A quality control manager regulating an individual production process will, for example, often want to monitor several parameters to ensure they stay within pre-defined limits. If some process does start to go wrong then the manager will need to know as soon as possible to allow corrective action to be taken at the earliest opportunity.

A graphical representation often highlights abnormal behavior most effectively and serves as a record for the behaviour of a process over a period of time. Quality Control Charts are often used as part of a quality control process to maintain and record the performance of a system over a period of time. If the process stays within the pre-defined limits then the process is said to be under control. By contrast, if the process goes outside the limits imposed on the system, it is said to be out of control.

Shewart Charts are designed to graphically plot a process to determine whether or not it remains under control and to help bring in remedial action to get the process back under control if necessary. This requires some appropriate variable to be recorded periodically. If an observation falls outside the outer limits this indicates that the process is out of control and action is required. If two or more consecutive readings fall between the warning limits and the outer limits then action should be taken.

The inner or warning set of control limits are set to represent $\pm 2 \sigma$ or $\pm 2 s$. The outer set of control limits is set to represent $\pm 3 \sigma$ or $\pm 3 s$.

5.4.1 Quality Control Chart

Quality control is those step take to ensure that an analysis is under statistical control to distinguish between variation in a process resulting from common causes and variation resulting from special causes. It is a graphical display of process stability or instability over time. One goal of using a Control Chart is to achieve and maintain process stability. This consistency is characterized by a stream of data falling within control limits based on plus or minus 3 standard deviations (3 sigma) and plus or minus 2 standard deviations (2 sigma) from the mean value, \bar{x} , as shown below.

$$UCL = \bar{x} + 3 SD$$

$$UWL = \bar{x} + 2 SD$$

$$LWL = \bar{x} - 2 SD$$

$$LCL = \bar{x} - 3 SD$$

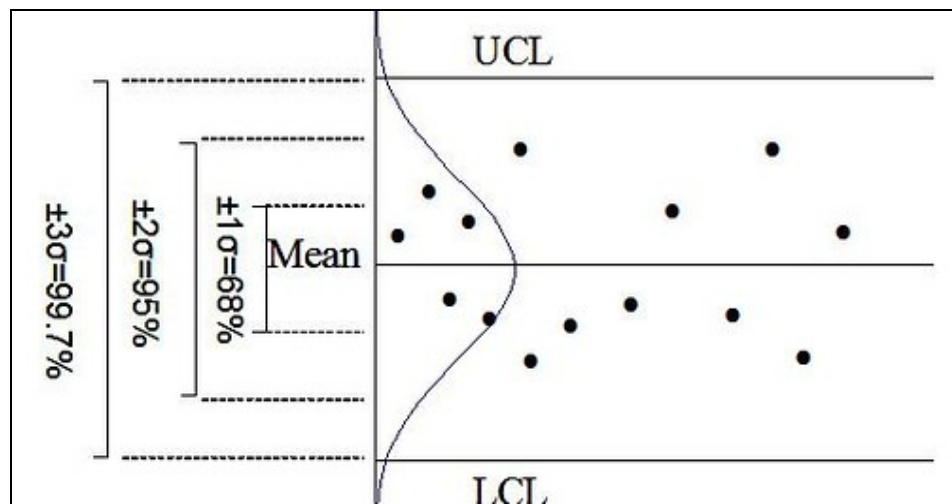


Figure 12 : Graphical demonstration of the QC Charts or Shewhart Chart.

The QC chart was constructed for determination of Hexavalent Chromium at concentration level of 0.05 mg/L of Cr^{6+} as shown in Figure 10. Every 10 samples will

run for one QC as to monitor for the performance of the analysis. The $\pm 2s$ or 2 sigma is the warning level and $\pm 3s$ or 3 sigma is the control level. If the QC range is out of specification, where it is out of control region, $\pm 3s$ or 3 sigma, then the analysis should be repeated as the QC is failed and out of control. If the QC range is fall out warning region, $\pm 2s$ or 2 sigma, the appropriate corrective action should be taken to monitor for the QC result.

5.5 Measurement Uncertainty

Measurement uncertainty is a statistical parameter which describes the possible fluctuations of the result of a measurement. It is not a mere repeatability but it is at least as high as the intra-laboratory reproducibility. It is a measure for the accuracy of the result. The measurement uncertainty defines the range containing the true value of the measurement. An estimation of the measurement's uncertainty is required for testing and calibration laboratories complying with ISO/IEC 17025. Error is defined as the difference between an individual result and the " true value" of the measurant. It is a single value whereas uncertainty take the form of a range.

The uncertainty estimation process involved four steps which are listed as below:

1. Step 1 - Specification of the measurand.

Write down a clear statement of what is being measured, including a mathematical model or equation between the measurand and the parameters upon which it depends.

2. Step 2 - Identification of the uncertainty sources. To create a list of the uncertainty sources for the method. An alternative method is using a cause and effect diagram or "fish bone" diagram.
3. Step 3 - Quantification of the uncertainty components. Measure or estimate the size of the uncertainty associated with each potential source of uncertainty identified.
4. Quantify combined uncertainty. To combine all the uncertainties of the uncertainty that make a significant contribution to the combined uncertainty using the law of error propagation. Using the combined uncertainty, calculate the expanded uncertainty.

From the fish bone diagram as shown in Appendix B is a very easy way to listing out all the uncertainty sources, showing how they relate to each other and indicating their influence on the uncertainty of the result. It also can avoid double counting of the sources and have a clear indication of the uncertainty sources.

From the precision study, the standard uncertainty of the precision is 0.0515 by pooled the RSD of the three spiked concentrations precision data of 0.005 mg/L, 0.05 mg/L and 0.25 mg/L of hexavalent chromium in deionized water. Meanwhile, the standard uncertainty of recovery is contributed from the method recovery and sample recovery.

The standard uncertainty from the method recovery, μR_m is 0.0583 by using the precision data of spiked 0.05 mg/L hexavalent chromium in deionized water which is the Quality Control level. The standard uncertainty from the sample recovery, μR_s is 0.0028 by using the precision data of spiked 0.05 mg/L hexavalent chromium in

seawater, industrial effluent and river water. As a result, the standard uncertainty of the recovery will be calculated by combined both the standard uncertainty of method recovery and sample recovery as shown in 3.2.1 b (ii). Thus, the standard uncertainty of the recovery is 0.0584.

Following the estimation of individual or groups of components of uncertainty and expressing them as standard uncertainties, the next step is to calculate the combined uncertainty . The combined relative standard uncertainty is found to be 0.0517. The expanded uncertainty is observed at 95% confidence level in which K is equal to 2, thus the expanded uncertainty will be 0.1034.

If the concentration of Hexavalent Chromium is 0.05 mg/L in the sample, the uncertainty of Hexavalent Chromium will be calculated by multiply the sample concentration with the expended uncertainty value. Therefore, at concentration 0.05 mg/L of Hexavalent Chromium in the sample, the uncertainty is 0.005 mg/L and this can be written as 0.050 ± 0.005 mg/L Hexavalent Chromium and the uncertainty is 10 % from the concentration of 0.05 mg/L. The acceptable value of the measurement uncertainty should be not more than 20% from the concentration value.

CHAPTER 6

CONCLUSION

The method for the determination of Hexavalent Chromium in seawater, river water and industrial effluent by colorimetric method is successfully develop and validated in the laboratory. The objective of this study was achieved. By referring to this study, the linearity of the calibration graph was established in which the calibration point is ranging from 0 mg/L to 0.25 mg/L.

This study is carried out by spiking various concentration level ranging from 0.005 mg/L, 0.05 mg/L and 0.25 mg/L in deionized water, seawater, river water and industrial effluent. The recovery obtained from the analysis result of the spiked samples are falled within 88% to 105%. The method detection limit (MDL) for determination of Hexavalent Chromium by using Discrete Analyzer is 0.005 mg/L or 5 ppb. Therefore, for the reporting value for the analytical result which is less than the MDL value, it should be reported less than 0.005mg/L or 5 ppb which the study of the ppb is achieved by using Discrete Analyzer.

CHAPTER 7

REFERENCES

American Public Health Association (APHA 3500- Cr B Colorimetric Method) Standard Methods For The Examination Of Water And Wastewater, 21st Edition, 2005 APHA, AWWA, WPCF, Standard methods for the examination of water and wastewater, American Public Health Association, American Water Works Association and Water Pollution Control Federation, Washington, USA, 14th edition, 1975.

'Chapter 6.4 Chromium Air Quality Guidelines'. (2000). WHO Regional Office for Europe, Copenhagen, Denmark. Second Edition.

David, H. (2000). Modern Analytical Chemistry. International Edition. Mc Graw -Hill Higher Education, Singapore.

Guide Manual: Water and Wastewater Analysis. Central Pollution Control Board. Ministry of Environment & Forests. A Government of India Organization.

Healthcare Product Comparison System, Clinical Chemistry Analyzers, Automated, Discrete October 2002

Laurie M., et al. (2012). 'State of the Science of Hexavalent Chromium in Drinking Water'. Water Research Foundation

Pedro, L. G. , et al. Analytical Method Validation. Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Brazil, Spain.

Robson, B. L. (2007). Hexavalent Chromium Analysis, Reduction And Stabilization In Cement And Concrete. University of Witwatersrand, Johannesburg.

Skovbjerg, L.L. (2005). ' Reduction of hexavalent chromium by green rust sulphate: determination of end product and reduction mechanism'. University of Copenhagen.

Ellison, S.L.R., M. Rosslein and A. Williams. (2000). Eurachem/ Citac Guide. Quantifying Uncertainty in Analytical Measurement. Second Edition.

Seamus, P. J. H. (2004). Analytical Chemistry. First Published. Oxford University Press, New York.

Veronika, R. M. "Measurement Uncertainty". *J. of Chromatography A*, 1158 (2007) 15-24.

Westco, Scientific Instruments, Inc. SmartChem 200 Method 518-04 (Rev: September 2008) Hexavalent Chromium.

WHO, Guidelines for drinking water quality, 3rd ed., chapter 8.7, January 21, 2004.

Zhitkovich, A. (2011). Chromium in Drinking Water: Sources, Metabolism, and Cancer Risks. *J. Chem. Res. Toxicol.*, 24, 1617–1629.

APPENDICES