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THROUGH THE DUST

DETERMINATION OF INSOLUBLE HEXAVALENT CHROMIUM IN AIRBORNE PARTICULATE MATTER

SGS Environmetal Services

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Abstract

This research is to investigate to what extend the NIOSH-7605 and ISO-16740 methods can be used to determine insoluble hexavalent chromium in air. The methods were tested and optimized on the extraction and sample preparation steps. After optimization the methods were validation on limit of detection (LOD) and quantification (LOQ), accuracy, reproducibility and expanded uncertainty.

NIOSH-7605 was found to have a LOD of 0.02 µg and a working range from 0.05-13.75 µg. ISO-16740 was found to have a LOD of 0.02 µg and a working range of 0.05-10 µg. Both methods were also validated in terms of accuracy, reproducibility and expanded uncertainty on levels of 0.40 µg and 4.00 µg of chromate.

Both NIOSH-7605 and ISO-16740 can be used to determine the amount of insoluble hexavalent chromium in air. It is recommended to use the NIOSH-7605 since the pH of test solution were the same as the mobile phase of the IC. The opposite is true for ISO-16740 which can have some interference with low standards and blanks.

Contents

1. Introduction

Chromium is a natural element which occurs in oxidation state -2 to +6. From these nine oxidation states, only the ground state 0, +2, +3 and +6 are commonly found in nature (Anderson, 1989). It is well known that chromium, in the +3-oxidation state, is an essential nutrient. It is required for the metabolism of fat and normal sugars. An insufficient intake of dietary chromium can lead to cardiovascular diseases and/or diabetes type II.

Hexavalent chromium, Cr(VI), is a strong oxidizing agent which is almost always bonded with oxygen (Mertz, 1969). This oxidation state of chromium is known as a human raspatory carcinogen and contact allergens (Barceloux, 1999; Das & Mishra, 2008; Sun, Brocato, & Costa, 2015; Von Burg & Lui, 1993). Long-time exposure to compounds which contain Cr(VI) can lead to an increased risk of developing lung cancer. Occupational exposure to these compounds may lead to nasal epithelia damage, asthma and damage to the skin (Occupational Safety and Health Administration, 2006).

Hexavalent chromium is used in many applications like corrosion prevention, wood preservation, metal finishing, plating, production of many pigments and in glassware cleaning solutions (Barceloux, 1999). During these operations workers are exposed to airborne hexavalent chromium particles. Since Cr(VI) is so toxic, there is a desire for a method to measure the amount of airborne hexavalent chromium.

In 2003, the National Institute for Occupational Safety and Health (NIOSH) published a standard method for the determination of hexavalent chromium in the air by ion chromatography. In 2005, the International Organization for Standardization (ISO) published also a standard method for the same determination. Although these organisations made a standard for the same analysis, they are different from each other.

SGS currently determines the amount of hexavalent chromium in air with a method which is on the standard method from NIOSH. However, this method is not yet validated for the SGS lab in ‘s Gravenpolder. This research is based on the standard analytical method of two organisations; the International Organization for Standardization (ISO) and the National Institute for Occupational Safety and Health (NIOSH). These organisations both have standards for the determination of hexavalent chromium in air. For the ISO it is their standard ISO-16740: Workplace air – Determination of hexavalent chromium in airborne particulate matter – Method by ion chromatography and spectrophotometric measurement using diphenyl carbazide (International Organization for Standardization, 2005), and for the NIOSH their standard 7605: Chromium, Hexavalent by Ion Chromatography (National Institute for Occupational Safety and Health, 2003).

For this project the research question is: To what extend can hexavalent chromium be measured in airborne matter according to NIOSH-7605 and/or ISO-16740? During the internship these two methods will be compared, optimised and validated. The methods will be validated on limit of detection, limit of quantification, linear range, reproducibility, trueness and expanded uncertainty.

2.Theoretical framework

2.1 Chromium occurrence and health

Of all the elements in the earth's crust chromium is the sixths most abundant. The element is found in the mineral crocoite, which contains lead(II) chromate ($PbCrO_4$) (Bencko, 1985). Contact with chromium compounds by humans happens mostly during industrial processes, like ore processing, metal plating, welding and spray painting. Chromium appears in nature in various forms and oxidation states. The trivalent state of chromium is considered as the most stable in contrast to the hexavalent state, which is a strong oxidizing agent.

The trivalent form is an essential nutrient for humans. This oxidation state is found in many food products such as broccoli, grape juice, potatoes and different kinds of meat (National Institutes of Health, 2020). Chromium(III) used for the metabolism of insulin, the formation of glucose tolerance factor (Barceloux, 1999) and the control of metabolism of sugars and fats (Anderson, 1989). It is suggested that an adequate daily intake of chromium(III) is 50-200 µg (Iyengar, 1989). Any deficiency on chromium intake can lead to impaired glucose tolerance, elevated cholesterol and triglycerides, shaking, drowsiness and blurred vision (Anderson, 1989).

In contrast to the trivalent state is hexavalent chromium (Cr(VI)) highly toxic and a confirmed carcinogen. Depending on the oxidizing and corrosive properties of the compound, a lethal dose of Cr(VI) can range from 1-3 grams of the compound. Some compounds containing dichromate have a lower lethal dose (0.5-0.8 grams). When in contact with the skin the Cr(VI) can develop allergic dermatitis and irritation of the skin. Contact can also lead to oxidation of the DNA, which leads to mutations. Long term exposure can result in asthma, liver damage, abdominal pain, kidney failure, different kinds of cancer, bloody diarrhea, coma and death (Barceloux, 1999; Bencko, 1985; Das & Mishra, 2008; Mertz, 1969; McNeill, McLean, Parks, & Edwards, 2012; Sun, Brocato, & Costa, 2015).

Compounds containing Cr(VI) come into the body via inhalation, ingestion and skin contact. Before Cr(VI) can enter the blood or lymphatic moist, it must be dissolved in either gastric juice when ingested, water in the lungs when inhaled and body sweat when in contact with skin. When Cr(VI) is dissolved it can easily be converted to trivalent chromium. When this happens outside a body cell, there is no harm (Netherlands National Institute for Public Health and the Environment, 2015). However, when the compound enters an body cell via anionic transporters the Cr(VI) can oxidize parts of the DNA which can eventually lead to mutations, cancer of the liver, lungs, stomach (Sun, Brocato, & Costa, 2015), nose and nasal cavity cancer, chronic obstructive pulmonary disease (COPD), perforation of the nasal septum and larynx cancer (Rijksinstituut voor Volksgezondheid en Milieu, 2020).

2.2 Limit values

Airborne particulate matter containing Cr(VI) is variable in toxicity, based on the solubility of the compound. The threshold limit value (TLV) is the American limit value of different chromium compounds and can range from 0.01 mg m^{-3} for insoluble Cr(VI) compounds to 0.5 mg m^{-3} for metal and Cr(III) compounds. Insoluble Cr(VI) compounds are the most toxic in airborne material. This can even reach as low as 0.5 µg m^{-3} for strontium chromate (American Conference of Governmental Industrial Hygienists, 2012).

In November 2016 the Ministry of Social Affairs and Employment in the Netherlands published updated limit values for Cr(VI) particulate matter in air. The new limit value was set to 0.001 mg m^{-3} for a time weighted average of eight hours. With this new rule, the old values for soluble and insoluble compound expired. The

ministry also set-up a prohibitive value of 1 mg m^{-3}. These came in effect on March 1st, 2017. The European Scientific Committee on Occupational Exposure Limits (SCOEL) set the limit value of Cr(VI) compounds at 0.005 mg m^{-3} for the entire European Union (Sociaal Economische Raad, 2020). In Belgium the current limit value is 0.01 mg m^{-3}. After the 17th of January 2025 there are new limit values that will come in effect and is set to 0.005 mg m^{-3}, the same value as for the European Union (Federale Overheidsdienst Werkgelegenheid, Arbeid en Sociaal Overleg, 2020). Germany and France have the same limit values for all Cr(VI) as the Netherlands at 0.001 mg m^3 (Institut fur Arbeitsschutze der Deutschen Gesetzlichen Unfallversicherung, 2019; L'Institut national de la recherche scientifique, 2016). Ireland has limit value for all Cr(VI) compounds of 0.005 mg m^{-3} (Health and Safety Authority, 2020). The United Kingdom has a special rule to the limit value of all Cr(VI) compounds. Normally it would be 0.01 mg m^{-3}, but if the source of the Cr(VI) is an industrial process than the limit value is 0.025 mg m^{-3} (Health and Safety Executive, 2020).

2.3 Structures and chemical equilibrium

Hexavalent chromium compounds are salts which contain the chromate, CrO_4^{2-}, or dichromate, $Cr_2O_7^{2-}$, anion (figure 1). These compounds are strongly oxidizing and in aqueous solution interconvertible. In a neutral aqueous solution, the chromate and dichromate anions are in chemical equilibrium, according to the following equation:

$$2CrO_4^{2-} + 2H^+ \rightleftharpoons Cr_2O_7^{2-} + H_2O \rightleftharpoons 2\,HCrO_4^- \qquad \text{(reaction 1)}$$

In the environment hexavalent chromium is only found as $HCrO_4^-$ when pH is below 6.5 and as chromate when above pH 6.5 (Regan, Dushaj, & Stinchfield, 2019). In figure 2, the Pourbaix diagram of chromium is shown. This diagram maps out all the possible stable equilibrium phases of hexavalent and trivalent chromium. As shown under neutral pH there is an equilibrium between $HCrO_4^{2-}$ and CrO_4^{2-}. This equilibrium follows the reaction as given in reaction 1 above.

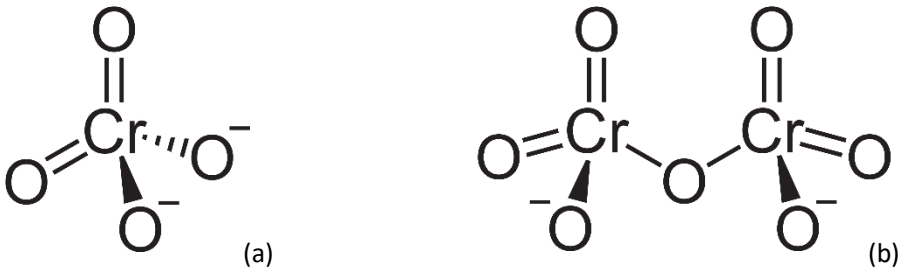


Figure 1: Structural formulas of chromate, CrO_4^{2-} (a), and dichromate, $Cr_2O_7^{2-}$ (b).

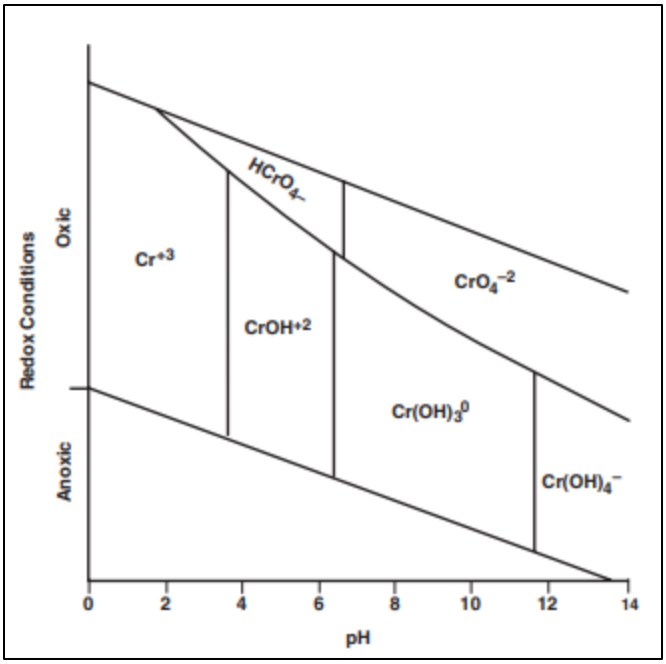


Figure 2: The Pourbaix diagram of chromium with the pH on the x-axis and the redox conditions on the y-axis. Shown are the species trivalent chromium (Cr^{3+}), chromium hydroxide ion ($CrOH^{2+}$), hydrogen chromate ion ($HCrO_4^-$), chromate ion (CrO_4^{2-}), chromium(III) hydroxide ($Cr(OH)_3$) and tertahydroxide chromium(III) ion ($Cr(OH)_4^-$) (McNeill, McLean, Parks, & Edwards, 2012).

According to Ashley et al. (2003), airborne Cr(VI) particles can be reduced to trivalent chromium, especially under acidic conditions. This is because the standard electrode potential of trivalent and hexavalent chromium is positive (reaction 2), therefor a reduction to Cr(III) is favoured:

$$HCrO_4^- + 7H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \qquad E^0 = +1.21\,V\ (pH\ 1) \qquad \text{(reaction 2)}$$

In an alkaline environment the equilibrium favours the stabilization of hexavalent chromium, according to reaction 3 below:

$$CrO_4^{2-} + 4H_2O + 3e^- \leftrightarrow Cr(OH)_2^+ + 6OH^- \qquad E^0 = -0.13\,V\ (pH\ 14) \qquad \text{(reaction 3)}$$

At pH 7 and above Cr(VI) dominates as an anionic ion while Cr(III) exists as a cation in the form of Cr^{3+}, $Cr(OH)^{2+}$ and $Cr(OH)_2^+$ (Pourbaix, 1974).

2.4 Sampling and sample preparation

The International Organisation of Standardization recognises two kinds of occupational exposure assessments, namely:

- Personal sampling: measures the personal exposure to hexavalent chromium by collection samples near the breathing zone.
- Static sampling: measure the hexavalent chromium level where personal sampling is not possible or to characterise the background level of a workplace

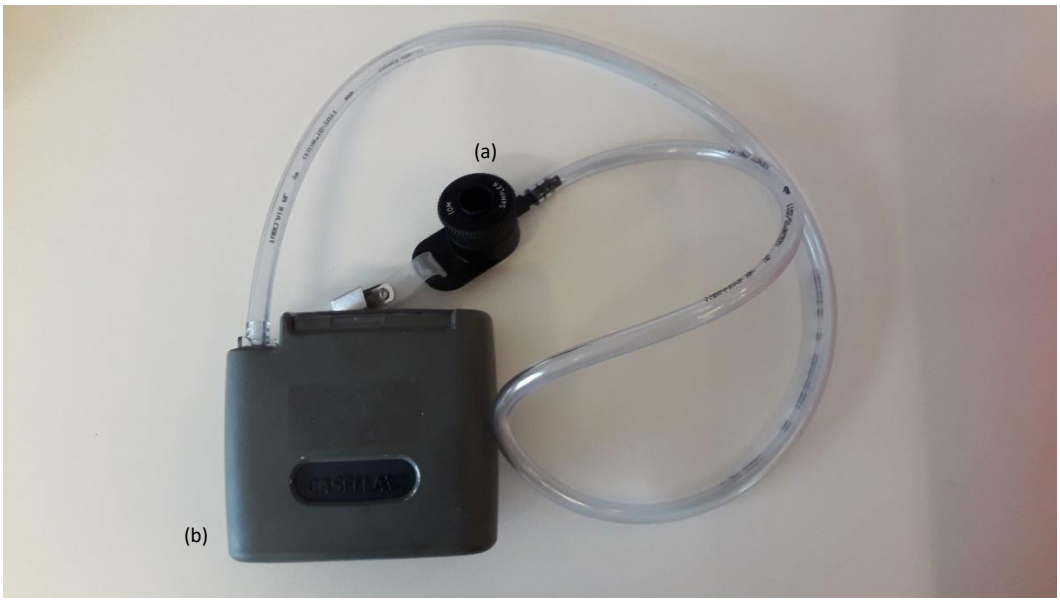


Figure 3: apparatus used for personal sampling with sampler (a) and air pump (b).

With personal sampling the person carries a personal sampler, see figure 3. The apparatus is a cassette or sampler (a) attached with a plastic tube to an air pump (b). Air filters are placed in the sampler and this is attached to the persons collar. This same apparatus can also be used for static sampling. Then the sampler is placed near an emission source or on another place were emission needs to be measured. (International Organization for Standardization, 2005).

The filter material used in sampling must be thought of carefully. When sampling, reduction of the Cr(VI) can occur by reactions on and with the filter material, which can result in lower recoveries. Glass fibre filters and cellulosic filters have binders in them which can lead to significant reduction of Cr(VI). Filter material that are suitable for sampling are polyvinylchloride (PVC), polyvinyl fluoride (PVF), polytetrafluoroethylene (PTFE), PVC- and PVF-acrylic copolymers and quartz fibre filters (Molina & Abell, 1987). Cr(VI) can also be reduced by other materials found in the matrix. These materials can be oxidizing agents in dust or organic particles. In acidic environments, like in chromic acid mist, the Cr(VI) is easily reduced to Cr(III), because it is unstable in an acidic environment (figure 2). Reduction could be prevented by soaking the filter material in a sodium hydroxide solution before sampling (Ashley, Howe, Demange, & Nygren, 2003).

Thomsen & Stern (1979) made a working scheme for separate determination of hexavalent chromium compounds. In this scheme the insoluble chromates on the filter are extracted with a sodium carbonate solution when heated on an hotplate. This protocol was further developed into ISO-16740 (Workplace air - Determination of hexavalent chromium in airborne particulate matter - Method by ion chromatography and spectrophotometric measurement using diphenyl carbazide) and NIOSH-7605 (Hexavalent chromium by ion chromatography). In these standards a buffer solution of 2% NaOH/3% Na_2CO_3 is used for extraction of the insoluble chromates. During the extraction the following reaction occurs:

$$XCrO_4 + {CO_3}^{2-} \rightleftharpoons XCO_3 + {CrO_4}^{2-} \qquad \text{(reaction 4)}$$

The presence of carbonate results in that the equilibrium shifts to the right. This makes that the insoluble chromates are dissolved in the solution. Any ions that can interfere with the reaction, like iron(II), have low solubility at high pH, so these cannot inhibit with the reaction (International Organization for Standardization, 2005). The use of an alkaline buffer can also result in an overestimation of Cr(VI), because during extraction Cr(III) can be oxidized. When Cr(III)-ions are in an alkaline environment, they form an hydroxo complex $[Cr(OH)_4]^-$:

$$Cr^{3+} + 3OH^- \leftrightarrow Cr(OH)_3 \qquad \text{(reaction 5)}$$

$$Cr(OH)_3 + OH^- \leftrightarrow [Cr(OH)_4]^- \qquad \text{(reaction 6)}$$

This complex can be oxidized by oxygen in the air to form hexavalent chromium in the form of a chromate-ion:

$$4[Cr(OH)_4]^- + 3O_2 + 4OH^- \rightarrow 4{CrO_4}^{2-} + 10H_2O \qquad \text{(reaction 7)}$$

This form of oxidation can be countered by the addition of magnesium hydroxide, which reacts with the trivalent chromium to form a precipitate (Zatka, 1985).

2.5 Separation by ion chromatography

It is necessary for many sample matrixes to separate and isolate the Cr(VI) prior to analysis. In some matrices other ions can interfere with the analysis by reducing Cr(VI) to Cr(III). For the spectrophotometric measurement it is crucial to extract any oxidizable metal ions, such as iron(II) ions. Any oxidizable metal ion present in the sample before analysis can reduce the Cr(VI) when it meets the acidic reagent.

The most used method of Cr(VI) separation is by ion chromatography (IC). This technique is based on the fact that, in a basic environment, Cr(VI) exists as an anionic species, while Cr(III) and Fe(II) exists as cations. Because of this the Cr(VI) can easily be separated from the ions which can interfere with the diphenylcarbazide reaction (Ashley, Howe, Demange, & Nygren, 2003). The mechanism of separation is based on exchanging between solute ions and charged sites on the stationary phase (Harris, 2010). The column contains alkyl quaternary ammonium as a functional group, which is a nitrogen cation surrounded by four alkyl groups, see figure 4 (Thermo Fisher Scientific , 2009). These groups classify as strongly basic and remain cationic at all pH values.

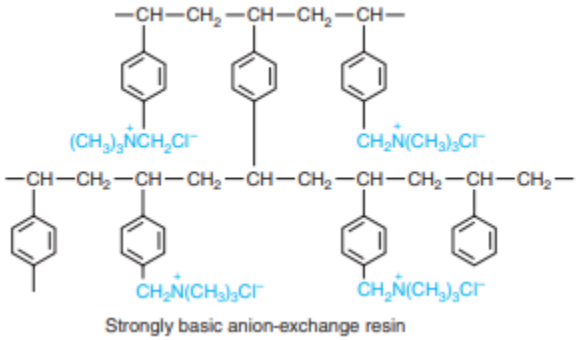


Figure 4: Structure of the ion-exchange resin used in the anion exchange column. The backbone is made of styrene-divinylbenzene cross-linked copolymer. The functional group is quaternary ammonium, represented as $(CH_3)3N^+CH_2Cl^-$ in blue (Harris, 2010).

2.6 Spectrophotometric measurement

The analysis of the Cr(VI) is done with a spectrophotometric measurement of a chromium-diphenylcarbazone-complex. After separation of the chromium by the ion chromatograph, the sample is subjected to an acidic solution of 1,5-diphenylcarbazide. The hexavalent chromium reacts with diphenylcarbazide and forms a purple colored complex which absorbs visible light at 530 nm (Thermo Fisher Scientific , 2009). Although the exact reaction mechanism is not known, suggestions on how the reaction works have been made.

According to Stencheva et al. (2013), only acidic solutions containing chromates react with 1,5-diphenylcarbazide. This reaction gives a magenta colored complex (λ = 530 nm). the complex is formed by the oxidation of 1,5-diphenylcarbazide (a.) by Cr(VI) to 1,5-diphenylcarbazone (b.) and then to 1,5-diphenylcarbadiazone, see figure 5. during this reaction Cr(VI) is reduced to trivalent chromium.

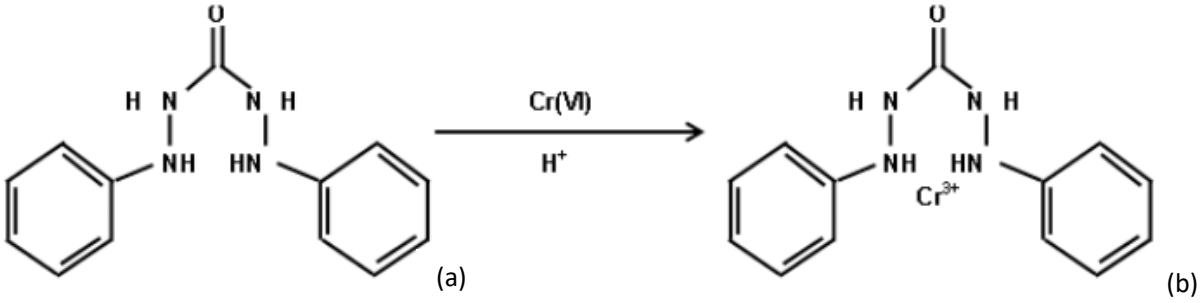


Figure 5: Reaction of 1,5-diphenylcarbazide (a) with hexavalent chromium under acidic conditions. This reaction forms a complex with trivalent chromium and 1,5-diphenylcarbazone (b) (Stancheva, Bogdanov, Georgiev, Hristov, & Markovska, 2013).

According to Feigl & Anger (1972), chromates react with diphenylcarbazide in strong acidic solutions to give a violet color. During this reaction the chromate oxidizes the diphenylcarbazide first to diphenylcarbazone and then to diphenylcarbadiazone, see figure 6. during this redox reaction the chromate ion is reduced to divalent chromium. This ion forms a complex with the enol form diphenylcarbazone.

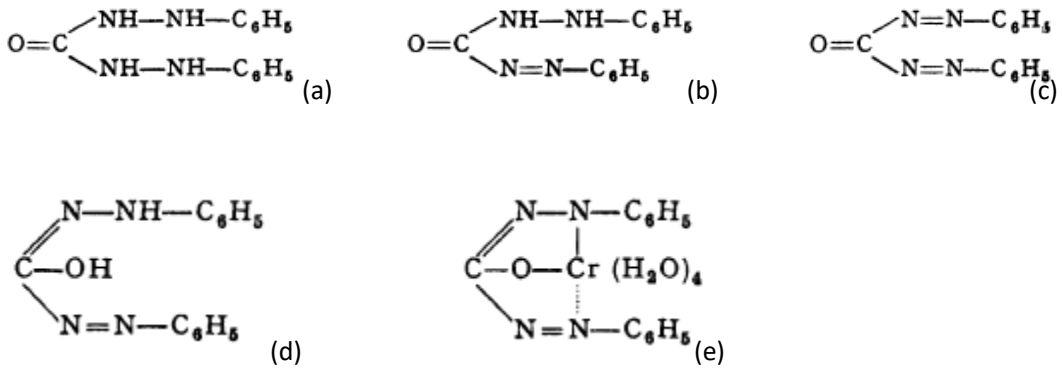


Figure 6: the oxidation of 1,5-diphenylcarbazide (a) to 1,5-diphenylcarbazone (b) and 1,5-diphenylcarbadiazone (c) by chromate and under acidic conditions. The keto form of diphenylcarbazone (d) reacts with divalent chromium to form the Cr-diphenylcarbazone complex (e) (Feigl & Anger, 1972).

This reaction can be interfered by the presence of various other ions and compound which can reduce Cr(VI). Any presence of molybdenum(VI), mercury(II), vanadium(V), copper(II) and iron(III) can result in a lower

determination of chromium since these ions can react with diphenylcarbazide and compete with Cr(VI) (Rowland, 1939).

2.7 Method validation

The process of proving that a certain analytical method is acceptable for its intended purpose is called method validation. With validation a couple of studies had been performed, these can include: specificity, accuracy, range, linearity, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness (Harris, 2010). For the validation of the ISO and NIOSH standards the focus is on validating blank level, LOD, LOQ, linearity, reproducibility, accuracy and expanded uncertainty.

2.7.1 Blank level

The blank level is determined by carrying regent blanks and laboratory blanks and analysing them. Reagent blanks contain only the reagents used for preparing the sample. Laboratory blanks are filters from the same batch, which have never left the laboratory. The response of the blanks is called the blank level (International Organization for Standardization, 2005).

2.7.2 Limit of detection

The limit of detection (LOD) is defined as the smallest amount or concentration of analyte of which can be detected with the analysis method. The detection limit can either be instrumental of method detection limit. The method detection limit can be determined by injecting a test solution near the anticipated detection limit ten times. The method detection limit is then calculated by multiplying the standard deviation (SD) of the mean concentrations found by three, see equation 1 (Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019; International Organization for Standardization, 2005).

$$Limit\ of\ detection\ (LOD) = 3 * SD \qquad \text{(equation 1)}$$

2.7.3 Limit of quantification

The limit of quantification (LOQ) is the smallest amount or concentration of analyte from which, with a stated accuracy and precision, can be quantified. In comparison to the LOD is the LOQ a quantitative border, instead the LOD is a qualitative border. The methods detection limit can be determined by fortifying several filters (n≥10) with a known amount of Cr(VI) near the anticipated detection limit. From the results of the test samples the LOQ can be calculated, according to equation 2 (Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019; International Organization for Standardization, 2005).

$$Limit\ of\ quantification\ (LOQ) = 6 * SD \qquad \text{(equation 2)}$$

2.7.4 Linearity

The linear range is the concentration range over which the calibration curve is linear and thus the response is proportional to the concentration. For determining the linearity, a calibration curve must be set-up with a minimum of six standards. Checked is whether the calibration point on the graph have a correlation $R^2 \geq 0.995$. The linearity is checked every day for these methods (International Organization for Standardization, 2005; National Institute for Occupational Safety and Health, 2003). Each point in the calibration curve is checked for its deviation in comparison to the calibration curve. This deviation is calculated according to equation 3.

$$\delta_{c,model} = \frac{X_{c,experimental} - X_{c,model}}{X_{c,model}} * 100\% \qquad \text{(equation 3)}$$

Where,
$\delta_{c,\ model}$ = percentual deviation of the experimental value in comparison to the calibration curve
$X_{c,\ experimental}$ = experimental response of the measurement of sample *c*
$X_{c,\ model}$ = model response calculated with the function of the calibration curve for sample *c*

Each individual calibration point can have a maximum deviation of 10%, except for the lowest point which can have a maximum deviation of 25%. When the deviation is greater than 10%, a new calibration curve must be set up by measuring standards again or by making new standards. When the deviation of each point is plotted in a graph the result must be a random distribution of the points. A systematic distribution of the points can suggest non-linearity. Any large deviation of points can lead to poor reproducibility and high uncertainty of the measurement (Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019).

2.7.5 Reproducibility

Reproducibility is a form of measuring precision when the circumstances under which the experiments are carried out are variable. These circumstances could be different lab spaces, different apparatus, other analysts and different batches of chemicals and reagents and carried out on other days. When reproducibility is determined in the same lab it is called intra-reproducibility. The minimum requirement for determining this is that there is a variation in time in which experiments are carried out. This means measuring on different days and with different batches.

There are two practical ways of determining intra-reproducibility, namely by caring out duplicate analysis of the different samples or by multiple analysis of the same sample. In the case of multiple analysis of the same sample, a sample is selected and measured a minimum of five times on different days. When there is a shortage of these samples, a sample can be created by spiking with the analyte. From these results the intra-reproducibility variance coefficient (CV_R) can be calculated, according to this equation:

$$CV_R = \sqrt{\frac{\sum_{i=1}^{n}\left(\frac{x_{i1}-x_{i2}}{0.5(x_{i1}+x_{i2})}\right)^2}{2n}} * 100\% \qquad \text{(equation 4)}$$

Where,
CV_R = intra-reproducibility variance coefficient
n = number of samples, n ≥ 5
x_{i1} = first result of the analysis on sample *i*
x_{i2} = second result of the analysis on sample *i*

2.7.6 Accuracy

With accuracy is investigated whether the results obtained by analysis are near to the true value. Demonstrating accuracy can in a couple of ways, like:

- Analysing a certified reference material in a similar matrix as the unknown sample. The tested method should find a value, within the precision of the apparatus, near the certified value in the reference material (International Organization for Standardization, 2005).
- Analysing a blank sample that is spiked with a known amount of analyte. These spiking values range from 0.5 to 1.5 times the expected sample concentration. matrix of these standards must be the same as the matrix of the unknown (National Institute for Occupational Safety and Health, 2003).

Each of these methods should give information about the accuracy. When analysing reference material, a minimum of five analysis must be carried out on the same sample under intra-reproducibility circumstances. When filters are spiked with a certain amount of chromate the recovery of the experiment can be calculated. With this calculation the found concentration for an individual experiment is compared with the true value of the spiked sample, according to the following equation:

$$T_{\Delta c,i} = \frac{X_{c+\Delta c,i} - X_{c,i}}{\Delta c} * 100\% \quad \text{(equation 5)}$$

Where,
$T_{\Delta c,i}$ = percentual recovery of the experiment *i* with spiked value *Δc*
$X_{c+\Delta c,i}$ = analysis result of experiment *i* for value *c* with spiked value *Δc*
$X_{c,i}$ = analysis result of experiment *i* for value *c*
Δc = spike value.

(Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019)

2.7.7 Expanded uncertainty

Expanded uncertainty is defined as an interval in which the true value of a certain measurement lies. In principal the expanded uncertainty covers every action that can be of influence on the result of a sample, such as conservation, preparation, measurement and analysis of the results. This uncertainty is determined by two factors, namely intra-reproducibility and accuracy of the method. The expanded uncertainty is not a performance characteristic, but a trait of the measurement (Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019).

To calculate the expanded uncertainty of the methods the following equations (6 to 10) must be solved in order. For every sample the accuracy is calculated using equation 5. Then from this value the bias of the measurement is calculated with equation 6.

$$b_i = T_{\Delta c,i} - 100 \quad \text{(equation 6)}$$

Where,
b_i = percentual bias for material *i*
$T_{\Delta c,i}$ = percentual recovery of the experiment *i* with spiked value *Δc*

The bias is calculated for each measurement and with these values the average bias of the experiments is calculated:

$$b = \frac{\sum_{i=1}^{n} b_i}{n} \quad \text{(equation 7)}$$

Where,
b = average bias of *n* experiments for material *i*
b_i = percentual bias for material *i*
n = number of experiments

The bias of a single experiment and the average bias of all the experiment are used to calculate the standard uncertainty of the average bias, according to equation 8.

$$u_{bias} = \frac{1}{\sqrt{n}} \sqrt{\frac{\sum_{i=1}^{n} (b_i - b)^2}{n-1}} \quad \text{(equation 8)}$$

Where,
u_{bias} = the standard uncertainty of the average bias
b = average bias of *n* experiments for material *i*
b_i = percentual bias for material *i*
n = number of materials

From the experiment the intra-reproducibility variance coefficient is calculated, but with a correction factor, according to equation 9.

$$CV_{Rw} = \frac{1}{\sqrt{2}}\sqrt{\frac{\sum_{i=1}^{n}\left(\frac{x_{i1}-x_{i1}}{0.5(x_{i1}+x_{i2})}\right)^2}{n}} * 100(\%) \quad \text{(equation 9)}$$

Where,
CV_{Rw} = intra-reproducibility variance coefficient
n = number of samples, n ≥ 5
x_{i1} = first result of the analysis on sample *i*
x_{i2} = second result of the analysis on sample *i*

Finally, the average bias, standard uncertainty of the average bias and the intra-reproducibility variance coefficient are used to calculate the expanded uncertainty according to equation 10.

$$U = |b| + 2\sqrt{(CV_{Rw})^2 + (u_{bias})^2} \quad \text{(equation 10)}$$

Where,
U = expanded uncertainty for the analysis results, in %
b = average bias, in %
CV_{Rw} = Intra -reproducibility variance coefficient
u_{bias} = the standard uncertainty of the average bias

(Energie- en milieu-informatiesysteem voor het Vlaamse Gewest, 2019)

3. Materials and methods

3.1 Instrumentation and chromatographic conditions

The separation of Cr(VI) was done on a Dionex ICS-1600 ion chromatography system (Thermo Fisher Scientific, Breda, Netherlands) equipped with Chromeleon software, Dionex AS40 automated sampler, connected to a post-column reagent system with Dionex APX pump and Dionex 750 µL reaction coil. The column used was a Dionex ionpac AS7 rfic (250x4 mm I.D.) and two guard columns, Dionex ionpac NG1 (35x4 mm I.D.) and Dionex AG7 (50x4 mm I.D.). The column was operated at a temperature of 30.0 °C. The mobile phase consisted of a buffer solution, 250 mM ammonium sulfate/200 mM ammonium hydroxide in water with flow rate of 1.5 mL min^{-1}. The injection volume was for all samples 50 µL. The runtime for the analysis was 7.5 minutes. The post column flowrate was 0.7 mL min^{-1}. Measurement of the Cr(VI)-complex was done on a Dionex ultimate 3000 variable wavelength detector at λ = 530 nm. Elution of Cr(VI) was after 3.3 minutes.

3.2 Chemicals and reagents

3.2.1 Reagents for sample preparation and analysis

All chemicals were of analytical or HPLC grade. All reagent and solutions were prepared with 18.2 MΩ cm ultrapure water delivered by an Elix Advantage 15 Water Purification System (Merck, Darmstadt, Germany).

Post-column derivatizing reagent was made by adding 56 mL of concentrated sulphuric acid (Honeywell, Seelze, Germany) to 1 L of ultrapure water and let it cool down to room temperature. Then 1.000 g of 1,5-diphenylcarbazide (VWR, Leuven, Belgium) was dissolved in 200 mL methanol (VWR, Leuven, Belgium). This solution in methanol was added to the sulphuric acid and diluted to 2 L with ultrapure water.

Insoluble hexavalent chromium extraction solution was made by dissolving 40 g sodium hydroxide (Merck, Darmstadt, Germany) in approximately 1 L ultrapure water, then 60 g sodium carbonate (Merck, Darmstadt, Germany) was added and dissolved. The solution was diluted to 2 L with ultrapure water.

Mobile phase was made by dissolving 66.00 g ammonium sulphate (Merck, Darmstadt, Germany) is approximately 1 L ultrapure water, then 13 mL concentrated ammonium hydroxide solution (J.T. Baker, Deventer, Netherlands) was added. The solution was diluted to 2 L with ultrapure water.

Ammonium buffer solution was made by dissolving 330 g ammonium sulphate (Merck, Darmstadt, Germany) in approximately 500 mL ultrapure water. Then to this solution 65 mL of concentrated ammonium hydroxide (J.T. Baker, Deventer, Netherlands) is added. The solution is diluted to 1 L with ultrapure water

NIOSH solvent was made by adding 364 mL insoluble hexavalent chromium extraction solution and 182 mL ammonium buffer to a 2 L polypropylene bottle and diluted to 2 L with ultrapure water.

ISO solvent was made by adding 1 L insoluble hexavalent chromium extraction solution and 1 L ammonium buffer to a 2 L polypropylene bottle.

3.2.2 Standard preparation

The stock solution of 1000 mg L^{-1} $Cr_2O_4^{2-}$ was prepared by dissolving 2.829 g dried $K_2Cr_2O_4$ (J.T. Baker, Leuven, Belgium) in water and diluted to 100 mL. The standard stock solution was made by diluted the stock solution 1:100 in either NIOSH or ISO solvent according to which calibration standards were made. The calibration stock

solution was made by dissolving this solution 1:10. From the calibration stock dependent stock calibration standards were made. NIOSH standards had concentrations of 0, 0.02, 0.21, 0.48, 0.96, 1.38, 2.06, 2.75, 5.50, 8.25, 11.00 and 13.75 µg chromate and were made by pipetting 0, 0.035, 0.375, 0.875, 1.75, 2.50, 3.75, 5.00, 10.00, 15.00, 20.00 and 25.00 mL of the NIOSH calibration stock into a 50 mL volumetric flask and diluted to 50 mL with NIOSH solvent. ISO calibration standards had concentrations of 0, 0.02, 0.05, 0.10, 0.50, 1.00, 2.00, 4.00, 6.00, 8.00 and 10.00 µg chromate and were made by adding 0, 0.05, 0.125, 0.25, 1.25, 2.50, 5.00, 10.00, 15.00, 20.00 and 25.00 mL of the ISO calibration stock to a 50 mL volumetric flask and were diluted to 50 mL with ISO solvent.

For the two methods were also 50 µg L^{-1} independent calibration standards made with potassium dichromate from Fluka (Sigma Aldrich, Steinheim, Germany). The independent NIOSH standard had a concentration of 1.38 µg and was made by pipetting 2.50 mL of the independent calibration stock in a 50 mL volumetric flask and diluted to 50 mL with NIOSH solvent. The independent ISO standard had a concentration of 1.00 µg and was made by pipetting 2.50 mL of the independent calibration stock in a 50 mL volumetric flask and diluted to 50 mL with ISO solvent.

The insoluble hexavalent chromium spiking standards were made by dissolving 11.2 mg lead(II)chromate (Alfa Aeser, Karlsruhe, Germany) in insoluble hexavalent chromium extraction solution and diluted to 100 mL. From this 20 mg L^{-1} CrO_4^- standards two other spiking standards made with concentrations of 2 and 0.1 mg L^{-1} by pipetting 10 and 2 mL into a 100 mL volumetric flask and diluted to 100 mL with insoluble hexavalent chromium extraction solution.

3.3 Specification of samples

For the determination of hexavalent chromium in the air, airborne particulate matter was collected on a 25mm PVC filter with pore size 5.0 µm using an IOM Sampler head connected to an Apex2 Personal Sampling Pump for (Casella, Bedford, United Kingdom) or a Gilian GilAir Plus (Sensidyne, St. Petersburg, United States). Samples are stored in a plastic cup with 5 mL extraction solution prior to extraction.

3.4 Sample preparation

The validated version of the working methods is given in section 3.4.1 and 3.4.2. The original working method of both the NIOSH and ISO standards are stated in appendix II and III.

3.4.1 According to ISO-16740

The filter was transferred to a 50 mL graduated polypropylene tube (Greiner Bio-One, Kremsmünster, Austria) with plastic forceps. Then 5 mL of the insoluble hexavalent chromium extraction solution is added, and the lid is closed. The tubes are sonicated in an ultrasonic bath for one hour. The solution is diluted to 10 mL with insoluble hexavalent chromium extraction solution and 10 mL of the ammonium buffer is added. Sample solutions are filtered prior to analysis.

3.4.2 According to NIOSH-7605

The filter was transferred to a 50 mL graduated polypropylene tube with plastic forceps. Then 5 mL of the insoluble hexavalent chromium extraction solution is added, and the lid is closed. The tubes are sonicated in an ultrasonic bath for one hour. The solution is diluted to 25 mL with water and then 2.5 mL of the ammonium buffer is added. Sample solutions are filtered prior to analysis.

3.4 Method validation

3.4.1 LOD and LOQ

The methods LOD and LOQ were determined by spiking ten filters with and standard solution near the LOD. The standard used is a solution of lead(II) chromate with a concentration of 100 µg L^{-1} for the ISO and NIOSH-method for an absolute chromate amount on the filter of 0.02 µg. The filters are loaded into the sampling devices and the pumps are set to 1.00 L min^{-1} and set to run for four hours for a total air volume of 240 L. The filters of both methods are prepared according to the sample preparation method and test solutions are made for analysis. From the IC analysis the LOD and LOQ are calculated according to equation 1 and 2 in the theoretical framework.

3.4.2 Linearity

For measuring the linearity of the methods, two sets of calibration standards were made, according to section 3.2.2. Ten calibration standards were made ranging from 0.00 µg to 13.75 µg Cr(VI) for the NIOSH-method and 0.00 µg to 10.00 µg for the ISO method. These standards were prepared every week and injected into the system every day. From the peak area two calibration curves were made and the linear regression (R^2) is calculated using the function in Microsoft Excel. To evaluate the linearity, the deviation of each point was calculated according to equation 3 and plotted in a graph.

3.4.3 Reproducibility, accuracy and expanded uncertainty

Everyday a set of filters was spiked with a solution of lead(II)chromate in extraction solution. These filters were spiked with 0.40 and 4.00 µg chromate by pipetting 200 µL of a 2000 or 20,000 µg L^{-1} solution on it. Air was drawn through these filters for four hours with a flow rate of 1.00 L min^{-1} for a total air volume of 240 L. After the four hours the filter were brought over to a plastic tube and Cr(VI) is extracted from the according to the NIOSH or ISO-method. Samples of these were analyzed and the amount of Cr(VI) found is compared with the theoretical value. From these results the intra-reproducibility variance coefficient, accuracy and expanded uncertainty was calculated according to equations 4 to 10.

4. Results

4.1 Method optimization

4.1.1 Extraction method

During the method optimization was tested what the best method of extraction was. With these experiments a tube with insoluble hexavalent chromium extraction solution was heated near the boiling point using a water bad and an oven. During heating the temperature was measured every five minutes, see figure 2 in appendix V for the results. Heating the solution in the water bath did not warmed the solution enough to reach the boiling point, only to a maximum temperature of 80 °C. Heating in an oven was more successful and the solution reached a temperature of 100 °C.

The next experiment was to test whether any extraction solution is lost during extracting the hexavalent chromium for one hour at 100 °C. for this experiment five tubes where filled with extraction solution and weighed. These tubes where than heated at 100 °C or 109 °C in an oven for one hour and then cooled back to room temperature outside the oven before being weighed again. The mass after the extraction was subtracted from the mass before and this difference is the loss, results are shown in figure 1 of appendix V. the average mass loss of the tubes heated at 107 °C was around 3% and when heated at 100 °C less than 1%.

Another observation was done during the previous experiment which was that all tubes boiled when cooling down back to room temperature. Since the solution must not boil during extraction a solution had to be found. The same experiment was repeated, but the tubes where only heated at 100 °C and after the one-hour mark were cooled down to room temperature inside or outside the oven (plot not shown). There was no significant difference in mass loss found in this experiment since the average for both experiments were less than 1%. The tubes that cooled down inside the oven did not boil, ones that cooled down outside the oven did boil.

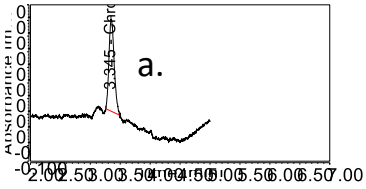
With this procedure of extraction. in an oven at 100 °C for one hour and cooling down inside the oven to room temperature, some blanco samples were tested. These blanco's contained 5 mL extraction solution and were extracted as stated above. After extraction these solutions were split into two groups, one for each method. To the NIOSH group 20 mL water was added and to the ISO group another 5 mL extraction solution before being injected into the IC system. The result showed small peaks at the retention time of Cr(VI) for both ISO and NIOSH methods, see figure 7a.

In order to make a real blanco without peak at the retention of Cr(VI) the extraction solution was purged with nitrogen gas. This step is suggested in the NIOSH method if high concentrations of trivalent chromium are expected. The purpose of adding nitrogen gas is to purge any oxygen out of the extraction tube so that any Cr(III) could not be oxidized (National Institute for Occupational Safety and Health, 2003). Before extraction the extraction solution and headspace above the liquid was purged with nitrogen gas for five minutes. After extraction and analysis, the peaks at the retention time of Cr(VI) where a lot smaller in peak area, see figure 7b.

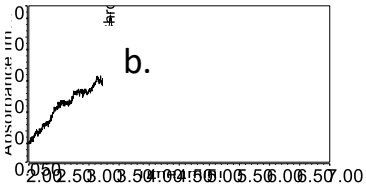
4.1.2 Possible contamination

To investigate the source of a possible contamination different methods have been tested. The chemicals used to make the insoluble hexavalent chromium solution were tested and new ones were ordered to test on hexavalent chromium. Also, a possible contamination could be in glasswork (International Organization for

Standardization, 2005), so this was washed with dilute nitric acid (1:10) and water before samples, solutions and calibration standards were made. The oven extraction method was switched for an extraction using an ultrasonic bath, as suggested in both methods as a second option. All these methods did not result in a real blanc sample.



Sample:	Blanc without nitrogen ISO
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm



Sample:	Blanc with nitrogen ISO
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm

*Figure 7: chromatograms of two measurements of blanco samples prepared according to the ISO-method. Sample A is not purged with nitrogen before extraction and has a peak area of 0.0298 mAU*min. Sample B is purged with nitrogen gas for five minutes before extraction and has a peak area of 0.0089 mAU*min.*

The only thing that was not yet tested for was the pH of the samples in comparison to the mobile phase. This was where the real problem lied. The pH of the extraction solution, mobile phase and samples after extraction

for NIOSH and ISO methods were tested and were found to be 13.6, 9.0, 13.1 and 13,6 respectively. The pH of the sample solution had to be lowered to around the pH of 9.0-9.5. This was done by adding an amount of ammonium buffer to the samples after extraction and addition of the water or extraction solution for NIOSH and ISO methods respectively. To reach the lower pH, 2.50 mL of the buffer had to be added to NIOSH samples and 10.00 mL to ISO samples.

A new protocol was made for both methods with the knowledge of the method optimization. From now on and during validation al samples would be extracted using an ultrasonic bath for one hour and after extraction 20,00 mL of water and 2.50 mL of ammonium buffer are added to NIOSH samples. For ISO samples 5,00 mL of extraction solution and 10,00 mL ammonium buffer are added after extraction.

Tests were done with both these new methods to check whether a good recovery was possible. For the NIOSH and ISO methods a set of filters were spiked with spiking standard to get 0.02, 0.40 and 4.00 µg chromate on the filter. These filters were extracted and prepared according to the new method and injected into the IC after a calibration, results of the calibration are shown in figure 8 . The chromatograms in figure 9 show that the lowest spiking on a filter is distinguishable from a blanco sample. The deviation on each point in the calibration curves is <10%. Spiked samples for both methods showed recoveries of Cr(VI) between 90-120%.

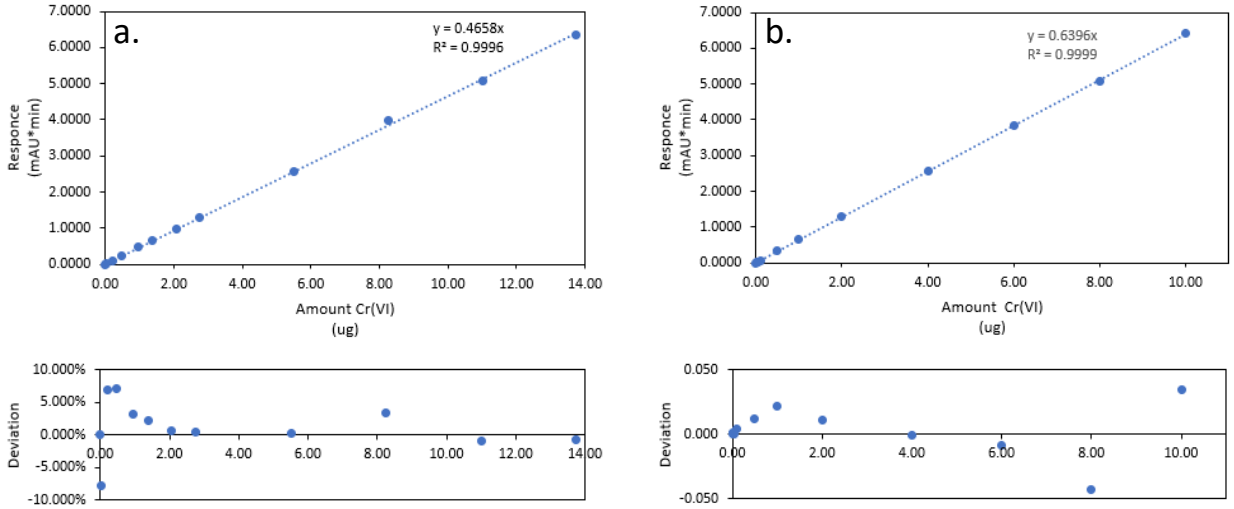
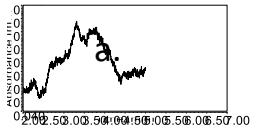
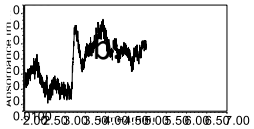


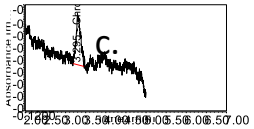
Figure 8: calibration curves and deviation for the NIOSH (a) and ISO (b) methods. The deviation of each individual calibration point in both lines are <10%.



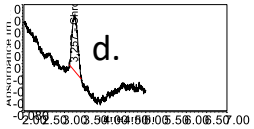
Sample:	Blanco NIOSH
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical
Column,	AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm



Sample:	Blanco ISO
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm



Sample:	Filter spiked at 0.02 ug NIOSH
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm



Sample:	Filter spiked at 0.02 ug ISO
Injection Volume:	50 µL
Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column, NG1 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2SO_4$/200 mM NH_4OH
Flow Rate:	1.5 mL min^{-1}
Postcolumn Reagent:	2mM diphenylcarbazide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.7 mL min^{-1}
Detector:	VIS, 530 nm

Figure 9: chromatograms of four samples with the new method for NIOSH and ISO. Two blanco injections were made for NIOSH (a.) and ISO (b.). Spiked filters were analyzed with a theoretical chromate amount of 0.02 µg on the filter. These filters were prepared according to NIOSH (c.) and ISO (d.) methods.

4.2 Method validation

4.2.1 Validation of NIOSH-7605

4.2.1.1 Linearity

To evaluate the linearity of the NIOSH a calibration was set up with eleven calibration standards ranging from 0 to 13,75 µg of chromate. These standards were injected every validation day before any other experiments were carried out. After analysis a calibration curve was set-up and checked for linearity using linear regression and evaluation of the residuals. The results of the ten obtained calibration curves are shown in figure 10a. The calibration curves over the ten validation days changed not much from shape or slope and the ten lines have no large deviation from each other. Tis small deviation is also seen in figure 10b where the average calibration line is shown. This line is made by taking the average value of each calibration point and calculating the standard deviation from these. The standard deviation is largest at the highest calibration standard, which is expected. The R^2-value of each calibration line was not lied between 0.9995 and 1.0000.

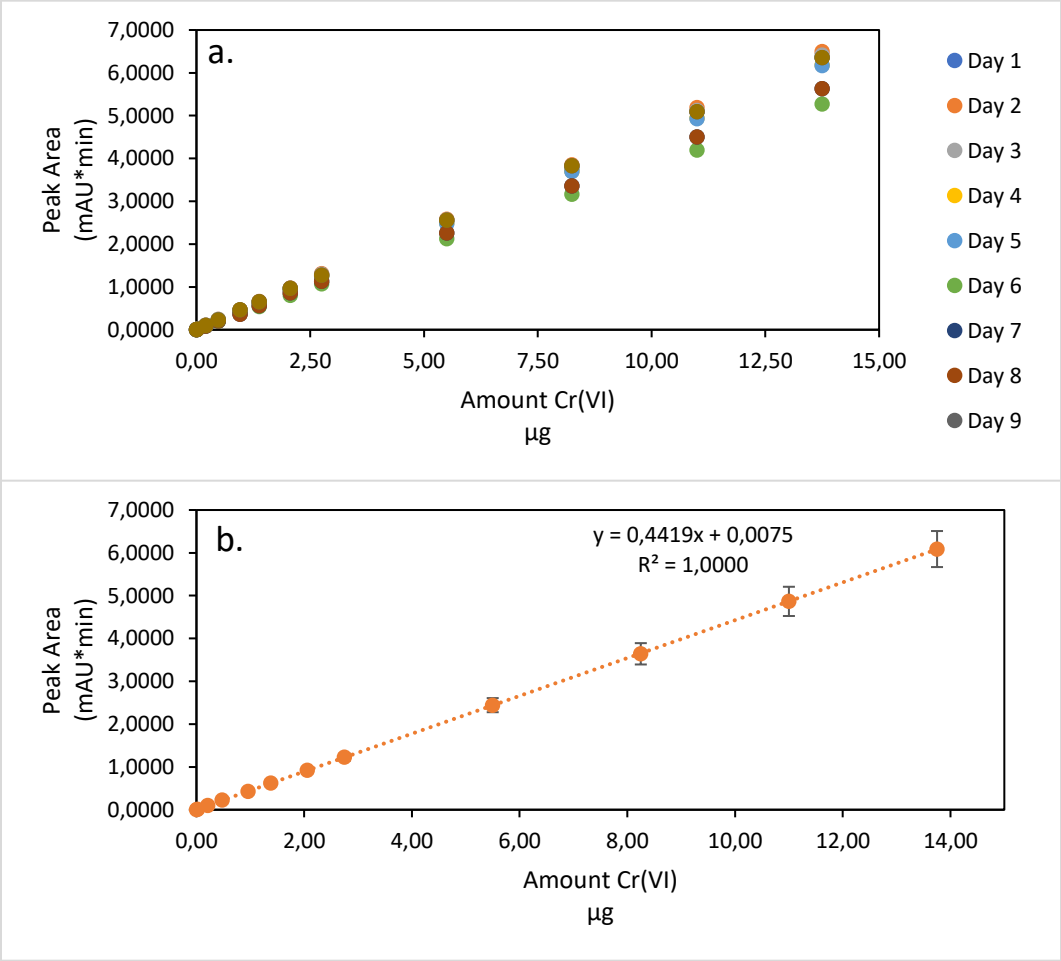


Figure 10: All calibration curves obtained over the ten-validation day for the NIOSH-method (A). Standards ranged from 0-13,75 µg chromate. The average of these point formed the regression curve with the standard deviation of each point (B)

4.2.1.2 Limit of detection

The methods limit of detection was determined by spiking ten filters over the period of ten days, one each day, with 200 µL of a 100 µg/L solution of lead(II)chromate for 0.02 µg of chromate on the filter. 240 L of air was drawn over the filters. The same was done with a filter spiked with 0.05 µg of chromate. The LOD and LOQ were calculated form the results of these spiking's and are shown in table 1. The standard deviation of the measurement was found to be very low with values of 0.002 and 0.006 for 0.02 µg and 0.05 µg respectively. This resulted in a LOD and LOQ for 0.02 µg of 0.005 µg and 0.009 µg respectively.

Table 1: Limit of detection (LOD) and Limit of Quantification (LOQ) found for the NIOSH-method. The used spiking levels were 0.02 µg and 0.05 µg of chromate on a filter. Average recovery was >100% with a low standard deviation resulting in a low LOD and LOQ on both levels.

Spiked Cr(VI) µg	Average recovery µg	Standard deviation	LOD µg	LOQ µg
0.02	104.6	0.002	0.005	0.009
0.05	111.2	0.006	0.019	0.038

4.2.1.3 Accuracy, reproducibility and uncertainty

To validate the accuracy, reproducibility and uncertainty filters were spiked with 0.40 µg and 4.00 µg chromate and analyzed, results are shown in table 2. The recovery was calculated and had to lie between 80-120%. The average recovery of the analysis was 102.8 and 104.2 % for the low and high spiking respectively. The relative standard deviation (RSD) of these results had to be lower than 15% in order to validate the accuracy. The found value of the RSD was 10.32 and 4.75 % for 0.40 µg and 4.00 µg respectively. The uncertainty of the measurement had to be lower than 50% and was found to be 24.48 and 14.12 % for low and high spiking respectively.

Table 2: Accuracy, reproducibility and uncertainty results of the method validation. Filters were spiked with 0.40 µg and 4.00 µg of chromate and analyzed. From these results the average recovery, relative standard deviation (RSD) and uncertainty were calculated

Spiked Cr(VI) µg	Average recovery %	RSD %	Uncertainty %
0.40	102.8	10.32	24.48
4.00	104.2	4.75	14.12

4.2.2 Validation of ISO-16740

4.2.2.1 Linearity

To evaluate the linearity of the ISO a calibration was set up with ten calibration standards ranging from 0 to 10 µg of chromate. These standards were injected every validation day before any other experiments were carried out. After analysis a calibration curve was set-up and checked for linearity using linear regression and evaluation of the residuals. The results of the ten obtained calibration curves are shown in figure 11a. The calibration curves over the ten validation days changed not much from shape or slope and the ten lines have no large deviation from each other. Tis small deviation is also seen in figure 11b where the average calibration line is shown. This line is made by taking the average value of each calibration point and calculating the standard deviation from these. The standard deviation is largest at the highest calibration standard, which is expected. The R^2-value of each calibration line was not lied between 0.9990 and 1.0000.

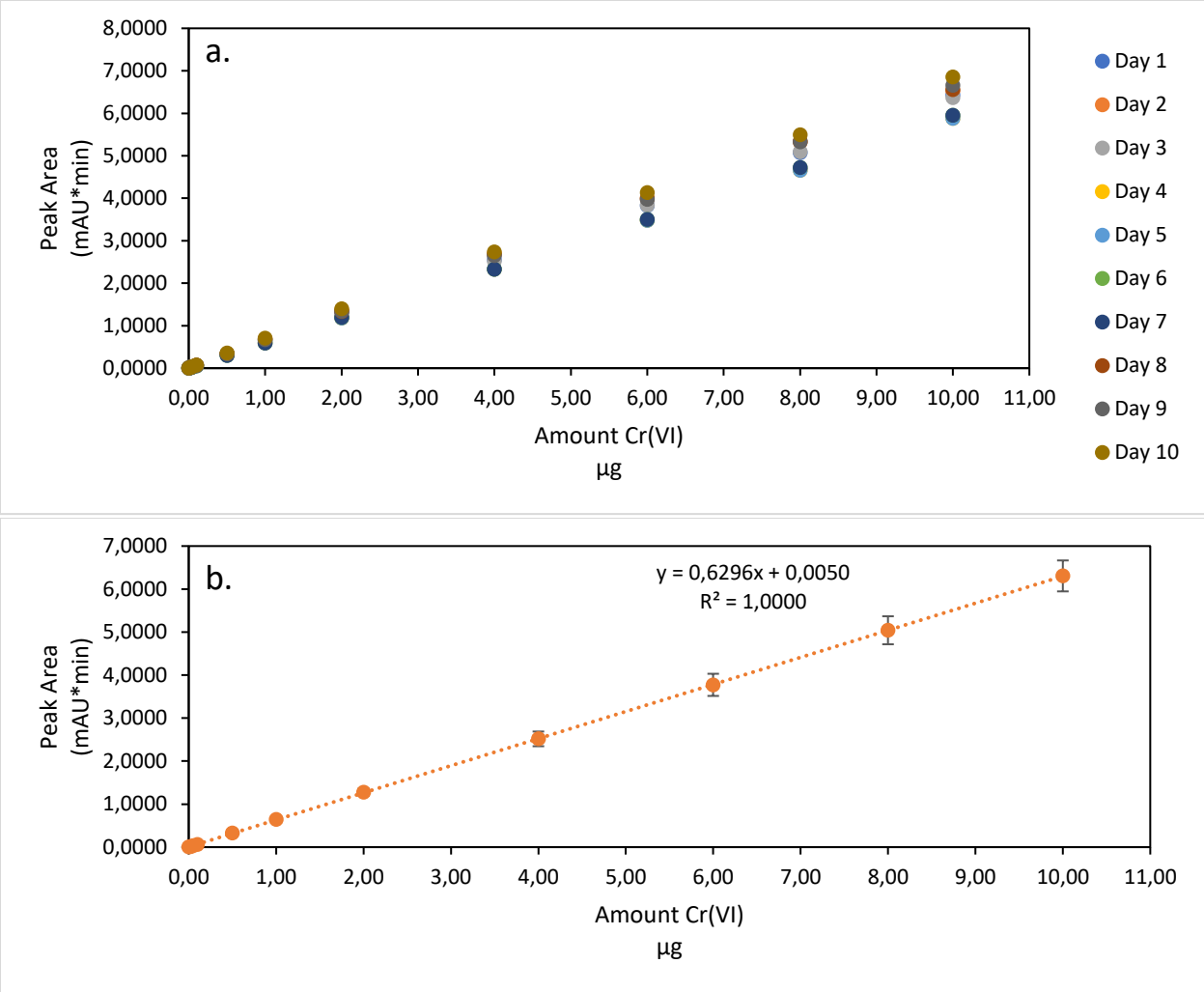


Figure 12: All calibration curves obtained over the ten-validation day for the ISO-method (A). Standards ranged from 0-10 µg chromate. The average of these point formed the regression curve with the standard deviation of each point (B)

4.2.2.2 Limit of detection

The methods limit of detection was determined by spiking ten filters over the period of ten days, one each day, with 200 µL of a 100 µg/L solution of lead(II)chromate for 0.02 µg of chromate on the filter. 240 L of air was drawn over the filters. The same was done with a filter spiked with 0.05 µg of chromate. The LOD and LOQ were calculated form the results of these spiking's and are shown in table 3. The standard deviation of the measurement was found to be very low with values of 0.007 and 0.009 for 0.02 µg and 0.05 µg respectively. This resulted in a LOD and LOQ for 0.02 µg of 0.020 µg and 0.040 µg respectively.

Table 3: Limit of detection (LOD) and Limit of Quantification (LOQ) found for the ISO-method. The used spiking levels were 0.02 µg and 0.05 µg of chromate on a filter. Average recovery was >100% with a low standard deviation resulting in a low LOD and LOQ on both levels.

Spiked Cr(VI) µg	Average recovery µg	Standard deviation	LOD µg	LOQ µg
0.40	132.3	0.007	0.020	0.040
4.00	108.1	0.009	0.026	0.053

4.2.2.3 Accuracy, reproducibility and uncertainty

To validate the accuracy, reproducibility and uncertainty filters were spiked with 0.40 µg and 4.00 µg chromate and analyzed, results are shown in table 4. The recovery was calculated and had to lie between 80-120%. The average recovery of the analysis was 107.1 and 106.0 % for the low and high spiking respectively. The relative standard deviation (RSD) of these results had to be lower than 15% in order to validate the accuracy. The found value of the RSD was 4.98 and 3.76 % for 0.40 µg and 4.00 µg respectively. The uncertainty of the measurement had to be lower than 50% and was found to be 17.59 and 13.88 % for low and high spiking respectively.

Table 4: Accuracy, reproducibility and uncertainty results of the method validation. Filters were spiked with 0.40 µg and 4.00 µg of chromate and analyzed. From these results the average recovery, relative standard deviation (RSD) and uncertainty were calculated

Spiked Cr(VI) µg	Average recovery %	RSD %	Uncertainty %
0.40	107.1	4.98	17.59
4.00	106.0	3.76	13.88

5. Discussion

5.1 Method optimization

The first thing that was done was the comparison of both methods to look for any differences. Both methods have roughly the same extraction and measurement method with only minor differences, such as the concentration of the mobile phase. A major difference in the methods is the sample preparation after extraction. For this reason, the methods could not be viewed as one method, but must be optimized and validated separately.

According to Ashley et al. (2003) the step that would give the most difficulties is the extraction, so this was investigated and tested a lot. Both methods recommend doing the extraction on a preheated hotplate. Since there are no hotplates available at SGS this was switch to a preheated oven instead. In the NIOSH method it is suggested to extract samples in a preheated oven, but to not boil the liquid. The reason to not boil the liquid was that, near the boiling point of water, Cr(III) could oxidize which results in an overestimation of Cr(VI) (Zatka, 1985). Thought was that this could be the reason for the small peaks in the sample blanco when testing for the oven extraction. Suggestions to overcome Cr(III) oxidation was to purge the extraction solution and headspace above it with nitrogen gas. With only small improvements when purging it thought that is was not primarily the oxidation of Cr(III).

The change in extraction method when going from an oven to an ultrasonic bath was made because the extraction in an ultrasonic bath performed as well as the oven extraction but at a lower temperature, according to the ISO method. The lower temperature also meant that the oxidation of Cr(III) if present was less favorable. This change was accepted by both method since it is named as an alternative to the oven and hotplate extraction.

5.2 Method validation

During method validation it was noticed that some blanc test solutions of the ISO-method showed some small peak on the retention time of Cr(VI). The peaks were the same area as a standard of 0.02 µg. These peaks were not because there was any hexavalent chromium present, but because of the pH of the test solutions was not the same as the pH of the mobile phase. This caused some interference in the measurement resulting in a peak at the retention time of hexavalent chromium. When this was noticed new calibration standards were made. This only happened twice during validation and only with the ISO-method.

Both methods are validated based on the results of the ten validation days. The results of all parameters meet the requirements set at the beginning of the validation.

6. Conclusion

In this research was investigated to what extend the methods NIOSH-7605 and ISO-16740 could analyze insoluble hexavalent chromium in airborne particulate matter. Both methods can detect Cr(VI) from 0.02 µg based on the limit of detection and quantify Cr(VI) from 0.05 µg according to the limit of quantification. This means that the working range for NIOSH-7605 is 0.05-13.75 µg of chromium and for ISO-16740 the working range is 0,05-10 µg. The methods were also validated on accuracy, reproducibility and uncertainty of the measurement. From the results can be concluded that both the NIOSH and ISO methods satisfy the requirements.

7. Recommendations

Since both methods are validated on LOD, LOQ, accuracy, reproducibility and uncertainty both NIOSH-7605 and ISO-16740 can be used to determine the insoluble hexavalent chromium in airborne particulate matter. It is recommended to use both methods and offer both to costumers.

For practical reasons it is recommended that the major method should be NIOSH-7605, since the pH of the test solution is the same as the pH of the mobile phase of the IC system. Because of this the blanc sample result in a negative result on Cr(VI). In the ISO method the pH is not the same as for the test solutions and mobile phase, so this could lead to some interference with low standards which could be contamination. However, this interference is not because Cr(VI) is present, but because the pH is not the same between test solution and mobile phase.

When both methods are used in routine work, SGS should participate in a proficiency testing program. This program test what the capabilities of the laboratory are in a certain research and testes whether the results are accurate.

In literature is found that some metal ion species could interfere with the recovery of hexavalent chromium. Therefor it is recommended to investigate the extend of this interference with the recovery of hexavalent chromium. The major metal ions that should be considered for this investigation are Fe(II) and Cu(II).

In short, both methods can be offered for the determination of Cr(VI), but NIOSH-7605 is favoured because of the pH of test solutions. SGS should participate in proficiency testing when the methods are used in routine analysis. The influence of metal ions should be investigated because they can interfere with the recovery of hexavalent chromium in the air.

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Appendices

Appendix I: specifications of hexavalent chromium analysis by Thermo Fischer on the DIONEX IonPax AS7 analytical column

5.11 Analysis of Hexavalent Chromium

The following chromatograms demonstrate the elution of hexavalent chromium using the AS7 column with postcolumn addition of diphenylcarbazide coupled with Visible detection.

Column:	IonPac AS7 (4-mm) Analytical Column, AG7 (4-mm) Guard Column
Eluent:	250 mM $(NH_4)_2 SO_4$/100 mM NH_4OH
Flow Rate:	1.5 mL/min.
Postcolumn Reagent:	2 mM Diphenylcarbizide 10% CH_3OH, 1 N H_2SO_4
PCR Flow Rate:	0.5 mL/min.
Detector:	VIS, 530 nm

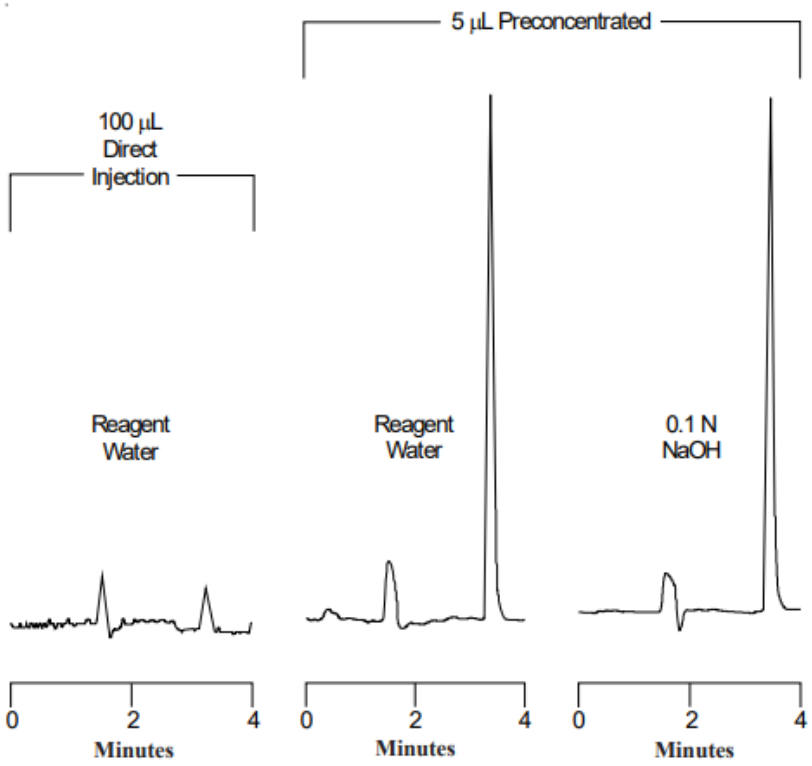

Figure 11
Analysis of Hexavalent Chromium
by Direct Injection and Preconcentration

Source: Thermo Fisher Scientific (2009)

Appendix II: analysis according to ISO-16740

Chemicals and reagents

- Sulfuric acid, conc. (98% w/w).
- Nitric acid, conc. (70% w/w).
- Nitric acid, diluted 1 to 10.
- Ammonium sulphate, (>99,5%).
- Ammonium hydroxide solution, conc. (29% w/w).
- Sodium carbonate, anhydrous (>99.9%).
- Sodium hydroxide, pellets (>99,5%).
- 1,5-diphenylcarbazide, (>98%).
- Methanol, HPLC grade.
- Potassium dichromate, (>99.9%).
- Insoluble hexavalent chromium extraction solution: sodium hydroxide (20 g/L)/sodium carbonate (30 g/L). Dissolve 20 g of sodium hydroxide pellets and 30 g of sodium carbonate in 250 mL of water, swirl to mix and allow to cool. Quantitatively transfer the solution to a 1-litre volumetric flask, dilute to the mark with water, stopper and mic thoroughly.
- Eluent concentrate: ammonium sulphate (2.0 mol/L)/ammonium hydroxide (1 mol/L). Dissolve 264 g of ammonium sulphate in approximately 500 mL of water. Quantitatively transfer the solution onto a 1-litre one-mark volumetric flask, add 65 mL of concentrated ammonium hydroxide and swirl to mic. Dilute to the mark with water, stopper and mix thoroughly. Store in a polypropylene bottle for a maximum period of one year.
- Eluent solution: ammonium sulphate (0.20 mol/L)/ammonium hydroxide (0.1 mol/L). add 100 mL of eluent concentrate to a 1-litre one-mark volumetric flask, dilute to the mark with water, stopper and mix thoroughly.
- Hexavalent chromium stock standard solution: 1000 mg/L hexavalent chromium stock standard solution. Dissolve 0.2828 g of potassium dichromate (previously dried to a constant mass at 110 °C and cooled in a desiccator) in water. Quantitatively transfer the solution into a 100 mL one-mark volumetric flask, dilute to the mark with water, stopper and mix thoroughly. Store in a polypropylene bottle for a maximum period of one year.
- Hexavalent chromium working standard solution: 10 mg/L of hexavalent chromium. Accurately pipette an appropriate volume, e.g. 10,0 mL of hexavalent chromium stock standard solution into a 1-litre one-mark volumetric flask, dilute to the mark with water, stopper and mix thoroughly. Prepare this solution fresh monthly.
- Diphenylcarbazide reagent solution. Add approximately 125 mL of water to a 250 mL one-mark volumetric flask. Slowly and carefully add 7 mL of concentrated sulfuric acid, swirl to mix and allow to cool. Dissolve 0.125 g of 1,5-diphenylcarbazide in 25 mL of methanol, quantitatively transfer the resulting solution into the volumetric flask, dilute to the mark with water, stopper and mix thoroughly. Prepare this solution fresh daily.

Material and equipment

- Samplers: polyvinyl chloride (PVC) membrane filters, pore size 5 µm or less.
- Sampling pumps, with an adjustable flow rate, capable of maintaining the selected flow rate to within ± 5% of the nominal value throughout the sampling period.
- Flat-tipped forceps, non-metallic, for loading and unloading filters into samplers.

- Filter transport cassettes or similar, if required, in which to transport samples to the laboratory.
- Disposable PVC gloves.
- One-mark volumetric flasks, of capacities between 10 mL and 1000 mL.
- One-mark pipettes.
- Polypropylene bottles, of capacities between 100 mL and 1000 mL.
- Polypropylene tubes, disposable, graduated, of capacity between 10 mL and 30 mL.
- Piston pipettes.
- Dispensers.
- Membrane filter, chemically inert.
- Ultrasonic bath, preferably with a timer, suitable for use in the ultrasonic extraction method.
- Ion chromatograph, having the following components: pump, autosampler, guard column, separator column, reagent delivery module, mixing tee and reaction coil, absorbance detector, computer and eluent reservoir.

Sample preparation by ultrasonic bath method

- Open the filter transport cassettes, sampler filter cassettes or samplers and transfer each filter into an individual, labelled tube using clean flat-tipped forceps.
- Add 5 mL of insoluble hexavalent chromium extraction solution to each beaker.
- Adjust the water level in the ultrasonic bath so that it is above the level of extraction solution in the tubes. Place the tubes in a suitable rack and load into the ultrasonic bath. Agitate the samples by applying ultrasound for 1 hour.
- After agitation, either
    - Unscrew the caps or remove the push-fit closures from the tubes and dilute each blank and sample solution to the 10 mL graduation of the tube with insoluble hexavalent chromium extraction solution. Reseal each tube with its screw cap or push-fit closure and mix thoroughly to produce the test solution, or
    - Quantitatively transfer each blank and sample solution to an individual, labelled, 10 mL volumetric flask, sensing out the tube and diluting to the mark with insoluble hexavalent chromium extraction solution. Stopper and mix thoroughly to produce the test solutions.

Calibration

- Prepare a minimum of six calibration solutions, including a calibration blank solution, to cover a concentration range from 0 µg/mL to 10 µg/mL of hexavalent chromium. Accurately pipette appropriate volumes of hexavalent chromium working standard solution into individual, labelled, 100 mL one-mark volumetric flasks. Dilute to the mark with insoluble hexavalent chromium extraction solution, stopper and mix thoroughly. Prepare the calibration solutions fresh daily.

Analysis

- Set up the ion chromatograph in accordance with manufacturer's instructions.
- Inject the calibration solutions into the ion chromatography system in order of increasing concentration and measure the absorbance of the hexavalent chromium peak for each calibration solution either in peak height or peak area mode.
- Use the instrument's computer to generate a calibration function using a linear regression. Repeat the calibration if the correlation coefficient, R^2, is not ≥ 0.999.
    - NOTE: If $R^2 < 0.999$, it might be possible to remove an erroneous calibration point and reprocess the data to obtain an acceptable calibration.

- Inject the blank and sample test solutions into the ion chromatography system and make absorbance measurements for each solution. Use the stored calibration function to determine the concentration of hexavalent chromium, in micrograms per litre.
- Analyse the calibration blank solution and a mid-range calibration solution after the initial calibration, and again after every ten test solutions. If the measured concentration of hexavalent chromium in the continuing calibration blank is greater than three times the instrumental detection limit, or if the measure concentration of hexavalent chromium in the continuing calibration verification has changed by more than ± 5%, take one of the following corrective measures. Either use the instrument software to correct for the sensitivity change (reslope facility), or suspend analysis and recalibrate the instrument. In either case, reanalyse the test solutions that were analysed during the period in which the sensitivity change occurred, or of that is not possible, reprocess the data to take account of the sensitivity change.
- Analyse reagent blank and laboratory blank solutions, and quality control solutions, and use the results to monitor the performance of the method.
- If concentrations of hexavalent chromium are found to be above the upper limit of the linear calibration range, dilute the test solutions in order to bring them within linear range and repeat the analysis. Add an appropriate volume of extraction solution when making dilutions, so that the diluted test solutions and the calibration solutions are matrix-matched, and record the dilution factor.
- Calculate the mean hexavalent chromium concentration of the blank test solutions, using the equation:

$$\rho_{Cr(VI)} = \frac{(\rho_{Cr(VI),1} * V_1 * F) - (\rho_{Cr(VI),0} * V_0)}{V}$$

Where

$\rho_{Cr(VI)}$: The calculated mass concentration of hexavalent chromium in the air sample, in micrograms per cubic metre,

$\rho_{Cr(VI),0}$: Is the mean mass concentration of hexavalent chromium in the field blank test solutions, in micrograms per litre,

$\rho_{Cr(VI),1}$: Is the mass concentration of hexavalent chromium in the sample test solution, in micrograms per litre,

V : Is the volume, in litres, of air sample,

V_0 : Is the volume, in millilitres, of the field blank test solution,

V_1 : Is the volume, in millilitres, of the sample test solution, and

F : Is the dilution factor (F = 1 in the absence of dilution).

(International Organization for Standardization, 2005)

Appendix III: analysis according to NIOSH-7605

Chemical and reagents

- Sulfuric acid, conc. (98% w/w).
- Ammonium hydroxide, conc. (28%).
- Ammonium sulphate monohydrate, reagent grade.
- Sodium carbonate, anhydrous.
- Sodium hydroxide, reagent grade.
- Methanol, HPLC grade.
- 1,5-diphenylcarbazide, reagent grade.
- Potassium dichromate or potassium chromate. Dry at 100 °C and store in a desiccator.
- Post-Column Derivatizing Reagent: Diphenylcarbazide solution. Dissolve 500 mg 1,5-diphenylcarbazide in 100 mL methanol. While stirring, add 500 mL water containing 28 mL of conc. Sulfuric acid. Dilute to a final volume of one litre with water. This reagent is stable for 4-5 days. Prepare in one-litre quantities as needed.
- Cr(VI) standard, 1000 mg/L. Dissolve 2.829 g potassium dichromate in deionized water to make one litre, or use commercially available solution. NOTE: 3.731 g K_2CrO_4 can also be used.
- Calibration stock solution, 1.0 µg/mL. Dilute 1000 µg/mL Cr(VI) standard 1:1000 with deionized water.
- Filter extraction solution, 2% NaOH-3% Na_2CO_3 in deionized water to make one litre of solution.
- Eluent (mobile phase); 250 mM ammonium sulphate/200 mM ammonium hydroxide. Dissolve 33 g ammonium sulphate in approximately 500 mL distilled water and add 6.5 mL conc. Ammonium hydroxide. Dilute to one litre with distilled water and mix.
- Nitrogen, pre-purified.

Materials and equipment

- Sampler: polyvinyl chloride (PVC) filter, 5.0-µm pore size, 37-mm diameter in polystyrene cassette filter holder.
- Personal sampling pump, 1 to 4 L/min, with flexible connecting tubing.
- Vials, scintillation, 20-,L glass, PTFE-lined screw cap.
- Forceps, non-metallic.
- Gloves, polypropylene or latex.
- Liquid chromatography apparatus consisting of autosampler; pump; NG1 (DIONEX Corp.) or equivalent guard column; HPIC-AS7, 4 x 250-mm (DIONEX Corp.) separator column (or equivalent); post-column reagent delivery system, 2.2m PEEK tubing mixing/reaction loop with 1 m in a water bath at 32 °C ± 3 °C; and UV detector.
- Filtration apparatus, PFTE luer-lock filter (Gelman IC Acrodisc or equivalent)/syringe.
- Beakers, borosilicate, 50-mL.
- Watch glass.
- Volumetric flasks, 25-, 100- and 1000-mL.
- Oven at 107 °C, not to exceed 115 °C. NOTE: hot plate can be used. An ultrasonic bath can be used instead of oven or hot plate.
- Micropipettes, 10-µL to 0.5-mL.
- Pipettes, TD 5-mL
- Bagged refrigerant
    - Clean all glassware with 1:1 HNO_3:H_2O and rinse thoroughly before use.

Sample preparation

- Don a clean pair of disposable plastic gloves(to prevent sample contamination). Using forceps, transfer the PVC filter to a 50-mL beaker, and add 5.0 mL filter extraction solution, 2% NaOH/3% Na_2CO_3. Start media blanks at this point.
    - NOTE 1: If significant amount of Cr(III) are expected to be present in the sample, either (a) degas the sodium hydroxide/ sodium carbonate extraction solution by bubbling nitrogen through it for 5 min. before proceeding, or (b) use a precipitation reagent.
    - NOTE 2: If only soluble chromates are of interest, use ammonium sulphate buffer in place of carbonate extraction solution.
- Cover the beaker with a watch glass and heat it to near the boiling point (100 °C to 115 °C) in an oven with occasional swirling for 45 min. Do not boil the solution. Longer heating times (up to 90 minutes) may be necessary for some samples (e.g., paint spray). Do not allow the solution to evaporate to dryness because hexavalent chromium may be lost due to reaction with the PVC filter and/or co-collected aerosol constituents. An indication that hexavalent chromium has been lost in this manner is a brown-coloured PVC filter.
    - NOTE: A hot plate, heater block, or ultrasonic bath can also be used for this step.
- Cool the solution and transfer it quantitively with distilled water rinses to a 25-mL volumetric flask. Bring to volume with distilled water.
    - NOTE: if the solution is cloudy, filter an aliquot through a PFTE luer lock filter attached to a syringe.
- Transfer an aliquot of the solution to the appropriate vial for the chromatograph's autosampler and analyse.

Calibration and quality control

- Calibrate daily with at least six working standards. Transfer 5 mL of extraction solution to each of a series of 25-mL volumetric flasks. Pipet known volumes (0-5 mL) of calibration stock solution (1.0 µg/mL) into the volumetric flasks. For higher standards, pipet 10 – 20 µL of the 1000 µg/mL concentrated stock and bring the volume to 25 mL with distilled water. These working standards contain 0 to 20 µg Cr(VI) per sample.
- Analyse the working standards together with blanks and samples.
- Prepare a calibration graph [response vs. µg (CrVI)]

Measurement

- Set the liquid chromatograph to manufacturer's recommendations and parameters. With a mobile phase flow rate of 1.0 mL/min., a post-column reagent flow rate of 0.7 mL/min., and a 2.2-m post-column tube, the derivative retention time should be approximately 3.7 – 4.7 minutes.
    - NOTE: If the instrument response for the samples is higher than the standards, dilute using a 1:5 dilution of extraction solution: water to maintain a constant ionic strength; repeat the analysis; and multiply the measured concentration by the appropriate dilution factor. Alternatively, inject a smaller volume and multiply by the appropriate factor.
- After the analysis is complete, flush the entire system with ASTM Type II water for at least one hour at 1.0 mL/min. with all columns on line. Remove the columns and continue flushing for an additional two hours. Flush the autosampler with several injections of water. Leaving the columns in line while the system is idle is not recommended.

Calculations

- From the calibration graph, determine the mass of Cr(VI) in each sample, W (µg), and in the average blank, B (µg).
- Calculate concentration, C (mg/m^3), of Cr(VI) in the air volume sampled, V (L):

$$C = \frac{W - B}{V}, mg/m^3$$

(National Institute for Occupational Safety and Health, 2003)

Appendix IV: calculations on the theoretical boiling point of the extraction solution.

Theoretical boiling point of the insoluble hexavalent chromium extraction solution

In this report the theoretical boiling point of the insoluble hexavalent chromium extraction solution is calculated using the formula for calculating the boiling-point elevation. Found was that the amount of dissolved salts gives a boiling-point elevation of 1.0 °C. This results in that the theoretical boiling point of the solution is 101.0 °C.

Boiling-point elevation is caused by the addition another compound to a liquid. This results in that the boiling point of the pure solvent is elevated. This phenomenon happens only when nonvolatile compounds are used, such as salts.

There are two factors which have influence on this phenomenon; the concentrations of the dissolved compound and the solvent. The elevation is not dependent of which compound is dissolved in the solvent, but how much. An increase in concentration is an increase in boiling point. If this happens with another compound in the same concentration, then the boiling point is the same.

The use of another solvent has influence on the boiling point as well. Each solvent has an ebullioscopic constant which describes the relation between the boiling-point elevation and the molarity.

The boiling-point elevation can be calculated with the following formula:

$$\Delta T = K \cdot \frac{M}{m} \cdot i$$

Where:

- ΔT: boiling-point elevation in °C
- K: ebullioscopic constant in °C kg mol^{-1}
- M: amount of dissolved compound in mol
- m: mass of the solvent in kg
- i: Van 't Hoff-factor

the Van 't Hoff-factor is a constant which describes the behavior of a solution. The factor is the ratio between the actual concentration and the amount in mol of dissolved compound.

Found in literate are the constant found for use in the formula's and the are for K 0.512 °C kg mol^{-1} and for i 2.5. The amount of salt dissolved in the solution is 0.004 mol. For the extraction is 5 g (0.005 kg) of extraction solution used. Filling this in the formula we get:

$$\Delta T = 0.512 \cdot \frac{0.004}{0.005} \cdot 2.5$$

Solving for ΔT gives:

$$\Delta T = 1.024\ °C$$

Adding to the boiling point of the solvent gives the theoretical boiling point T:

$$T = 1.024 + 100$$

$$T = 101.024\ °C$$

In conclusion, the theoretical boiling point of the insoluble hexavalent chromium extraction solution is around 101 °C.

Appendix V: additional tables and figures.

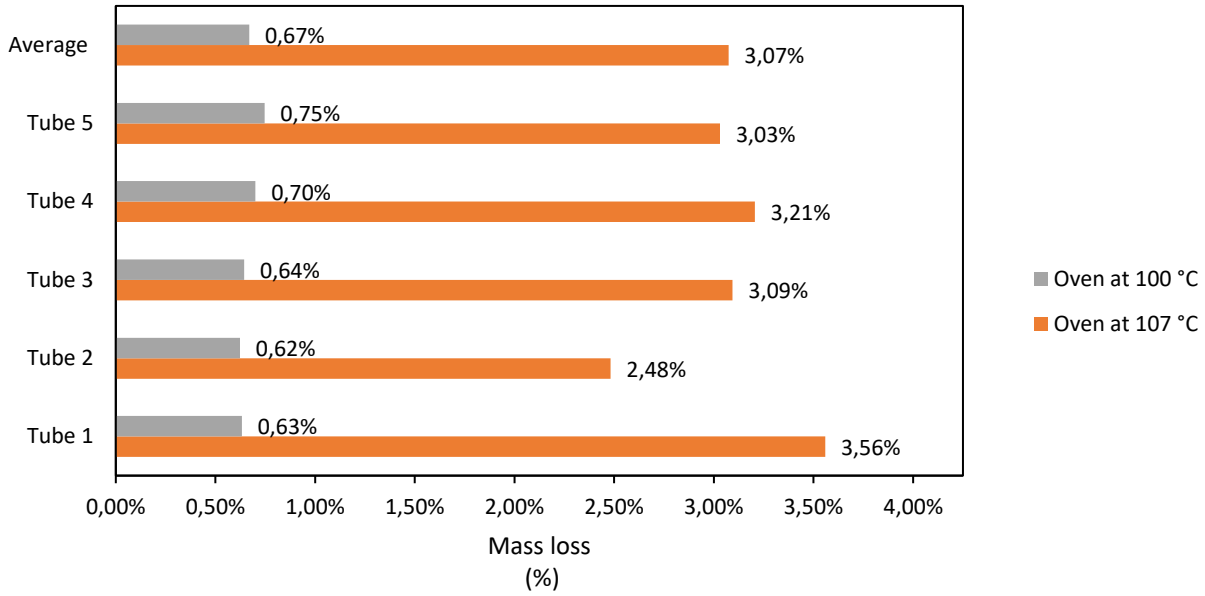

Figure 1: mass loss of the insoluble hexavalent chromium extraction solution during extraction in an oven at 100 °C and 107 °C for one hour and cooling back to room temperature outside the oven.

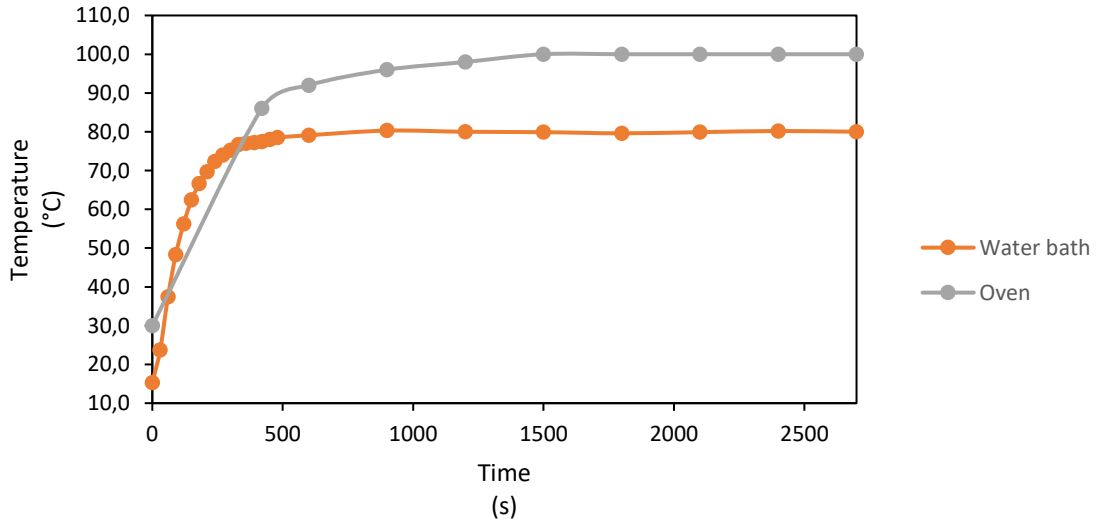

Figure 2: Temperature of the insoluble hexavalent chromium extraction solution when heated near the boiling point in a water bath (orange) and oven (grey).