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Department: Chemistry

Determination of Copper, Iron, Lead and Zinc Levels in Locally Produced Wines using Atomic Absorption Spectrometry (AAS)

A thesis submitted in partial fulfillment of the requirements for the Degree of Bachelor of Science in Chemistry

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Abstract

Wine is regularly consumed by many people, which can lead to the intake of various elements such as metals. However, if these metals are present in high concentration, they can negatively impact the wine quality and cause health issues. The aim of this research project was to determine the concentration of iron, copper, lead and zinc in locally produced wines, their respective juices and a foreign wine sample. This was done by pretreating the wine and juice samples with concentrated nitric acid, after which the solutions were concentrated. The concentrated solutions were subsequently diluted with deionized water to a volume of 100 mL. The absorbance of the solutions was measured using an Atomic Absorption Spectrometry (AAS) instrument and the data were further processed using Microsoft Excel. The concentration of iron in the samples determined by the calibration curve method was found to be ranging from 11.2 ± 0.8 ppm to 34.0 ±1.2 ppm. While, for the standard addition method, the concentration of iron was found to be range from 38.7 ± 0.1 ppm to 203 ± 0.138 ppm. The concentration of copper in the samples determined by the calibration curve method was found to be ranging from 0.150 ± 0.05 ppm to 1.69 ± 0.03 ppm. While for the standard addition method, the copper concentration was ranging from 0.231 ± 0.006 ppm to 2.67 ± 0.02 ppm. The concentration of zinc in the samples determined by the calibration curve method was found to be ranging from 1.37 ± 0.92 ppm to 6.60 ± 0.3 ppm. While for the standard addition method, the concentration range was 4.82 ± 2.53 ppm to 24.6 ± 1.4 ppm. For lead, all the absorbance values were negative, thus meaning that the concentration of lead was below the limit of detection of the instrument (0.03 ppm). The International Organization of Wine and Vine (O.I.V.) limit for iron, copper, lead and zinc is 10 ppm, 1 ppm, 0.15 ppm and 5 ppm, respectively. Iron and zinc in the locally produced wines exceeded the O.I.V. limit, while copper and lead were below the limits. When the wines were compared to their respective juices, it was found that the juices had a lower iron and zinc concentration than the wines. But, for copper, it was found that the concentration in the juices was higher than in the corresponding wines. When compared to a foreign wine, the levels of iron, copper and zinc found in the locally produced wines were overall higher than in the foreign wine. To remove some of the excess metals, it is recommended to have an ion exchange step in the winemaking process.

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Smriti Somai,

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List of Abbreviations and Acronyms

AAS	: Atomic Absorption Spectrometry
AES	: Atomic Emission Spectrometry
°C	: Degree Celsius
CV-AAS	: Cold Vapor Atomic Absorption Spectrometry
DNA	: Deoxyribonucleic acid
DPASV	: Differential Pulse Anodic Stripping Voltammetry
EU	: European Union
FAAS	: Flame Atomic Absorption Spectrometry
g	: Grams
GFAAS	: Graphite Furnace Atomic Absorption Spectrometer
h	: hour
IBC	: Intermediate Bulk Container
ICP-MS	: Inductively Coupled Plasma – Mass Spectrometer
ICP-OES	: Inductively Coupled Plasma – Optical Emission Spectrometer
L	: Liters
LOD	: Limit of Detection
LOQ	: Limit of Quantification
m	: minutes
mg	: milligrams
mL	: Milliliters
MS	: Microsoft
N	: number of measurements
nm	: nanometers
O.I.V.	: International Organization of Wine and Vine
ppb	: parts per billion
ppm	: Parts per million
s	: Standard Deviation
WHO	: World Health Organization
$\overline{x}$	: Mean
x_i	: sample

Specification of wine samples:

CR	: Carlo Rossi Cabernet Sauvignon wine
DD	: Dubru Du wine
DDS	: Dubru Du juice
KW	: Kruidenwijn
KWS	: Kruidenwijn juice
SA	: Standard addition spike
AC	: Accuracy spike

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Chapter 1: Introduction

1.1: General Overview

To stay alive, food and drinks are of prominent importance for human beings. It gives the human body the fuel to function properly. Apart from this, food and drinks are also consumed for pleasure, such as sweets and alcoholic drinks. Among alcoholic drinks, wine is one of the well-known ones because it is believed to positively impact health. A study done by Tariba (2011) states that consuming wine regularly can have several positive effects on human health. The most notable effect is lowered risk of coronary heart diseases. Regular wine consumption also leads to the intake of essential elements for humans such as iron (Fe), calcium (Ca), manganese (Mn), zinc (Zn), etc. (Bora, et al., 2017). According to a study done by Ibanez, Carreon- Alvarez, Barcena-Soto and Casillas (2008), metals are found in many alcoholic beverages such as beers, cognac, whiskeys, wines, etc. It is known that metals can have positive and negative health effects on consumers of alcoholic beverages.

Since ancient times, wine has been consumed regularly by many people around the world. Wine is an alcoholic drink made from fermented fruit (mostly grapes). In general, the average composition of the major components of wine are: water, ethanol, inorganic ions, organic acids, polyphenols, proteins, amino acids and polysaccharides. The alcohol content varies from 7% to 24%. (Pyrzynska, 2007). The overall chemical composition of wine is distinctive for each type of wine. It depends on several factors such as the type of fruit used for the wine, yeast used for the fermentation process, winemaking techniques, climate, etc. (Mitic, et al., 2014).

There are a variety of types of wines which can be divided based on the:

- Sugar content: dry (maximum of 4 g/L sugar content), demi-sec (maximum of 12 g/L sugar content), semi-sweet (maximum of 45 g/L sugar content), sweet (minimum of 45 g/L sugar content);
- Carbon Dioxide content: still (CO_2 concentration less than 4 g/L at 20°C), sparkling (between 3 g/L and 5 g/L at 20°C);
- Production techniques used during the process such as pretreatment of the fruit, different fermentation techniques, etc. (International Organization of Vine and Wine, 2021)

To ensure that the metal concentration in wines is at a safe level for consumption, commercially available wines are regularly tested for metals and the metal concentration has to be below or equal to the permissible values stated by the concerned health institution such as the World Health Organization (WHO), European Union (EU), International Organization of Vine and Wine (O.I.V). This is an international protocol, but in Suriname, the metal content of locally produced wines is not determined.

Therefore, this project focuses on the determination of iron, copper, lead and zinc in locally produced wines. The reason these 4 metals were chosen is because:

- Lead is one of the well-known and most described toxic metal. Because of the severity of lead poisoning, lead is one of the most prominent metals studied in food and beverages.
- Iron, copper and zinc are not necessarily toxic at lower levels, but at higher levels, it can cause poisoning and it- also affects the quality of the wine (taste, color, haze formation, etc.)
- The 4 metals can be detected with the AAS instrument available for students at the university. Because of the earlier practicals followed in the study program, experience has been gained in setting up an AAS experiment.

For this project iron, copper, lead and zinc were determined in locally produced wines and their respective juices. The metal concentration of a foreign wine was also determined. This was done to compare the metal content in locally produced wines and in a foreign wine sample to see if and how the metal content differs. Metals in wine can be analyzed by different methods such as electroanalytical

and spectroscopical methods, but for this study Atomic Absorption Spectroscopy (AAS) was used. The measurement of metal concentrations was done by 2 methods: the calibration curve method and the standard addition method.

1.2: Problem Statement

In Suriname, locally produced wines from several fruits and vegetables are commercially available. The metal content in these wines is unknown and could potentially have metal poisoning consequences. It was deemed necessary to determine the metal concentration in the locally produced wines to conclude whether or not the wines satisfy the standards of the International Organization of Vine and Wine (O.I.V.).

1.3: Central question and additional research questions

The central research question formulated for this project is: "***What is the concentration of copper, iron, lead and zinc in locally produced wines?***"

Additional research questions for this research project are formulated as follows:

1. Do the obtained results meet the permissible values stated by the O.I.V.?
2. What is the concentration of copper, iron, lead and zinc at the beginning of the wine production process?
3. What is the difference in the copper, iron, lead and zinc concentration at the beginning and the end of the production process?
4. How does the metal content from Surinamese wine differ from a foreign imported wine?

1.4: Relevance of the project

In Suriname, many companies produce homemade wines that are commercially available. On the labels of the bottles, no information regarding metal content is found. During an informal meetup with one of the producers of locally produced wines, it appeared that the wines were not tested for any metal concentrations. This may pose a danger to the users of these wines, as it is not known currently if the metal concentration exceeds the maximum acceptable limits. Therefore, the producer mentioned the wish to analyze some wine products for metal content. Thus, it is unclear if and what the metal concentrations are in locally produced wines of this producer. The results of this study can be used to determine if the locally produced wines pose a risk for human health.

Social relevance: Extensive consumption of locally produced fruit wines that are not examined for metals can lead to health effects. Determination of the metal concentration at the beginning and the end of the production process gives an indication of the metal content in the product and if the permissible values for the metals in the end product are exceeded. Doing so gives the producer and the consumer a sense of certainty and trust, which is beneficial for both parties.

Academic relevance: Metal content in locally produced wines in Suriname is not known. This project will provide research results that can provide insight into the metal content in locally produced wines and be a criterion that can be used for further research on winemaking in Suriname.

1.5: Outline of thesis

Chapter 2 gives the theoretical framework of the study and describes the method that was applied to measure the metal concentration in locally produced wines. In chapter 3, the materials and methods used to complete the practical part of this research project are described. Chapter 4 gives the results and these are discussed in chapter 5. Chapter 6 gives the conclusions and recommendations emerging from this study.

Chapter 2: Literature Review

2.1: Background information and composition of wines

Wine is an alcoholic drink that has been used for centuries. It is mostly made of fermented grapes, but other types of fruit and vegetables can also be used to produce wines. Wine is an excellent source of minerals, polyphenols, antioxidants etc. (Fermo et al., 2021). Apart from that, wine also contains several inorganic compounds such as alkaline and alkaline earth metals and transition metals. This makes wine a very chemically complex matrix. (Kostic, Mitic, Miletic, Despotovic, & Zarubica, 2010)

Winemaking involves different stages and practices depending on the fruit and the type of wine that is desired. In general, winemaking involves 6 steps that vary according to the wine type. These steps in sequential order are:

1. Harvest: the plant or fruit used to make the wine is harvested at an appropriate time.
2. Destemming and sorting: unwanted parts of the plants are removed and put away. The parts used to make the wine are crushed and then moved to the next step.
3. Fermentation: this step is where (in the absence of oxygen) yeast converts sugars into ethanol with carbon dioxide (CO_2) as a byproduct. The amount of yeast and sugar added to the fruitpulp depends on the type of wine and the desired flavor.
4. Press: the fermented fruit pulp is pressed to extract the resulting liquid.
5. Aging: the liquid is set to age (secondary fermentation). This is done to complete all the chemical reactions happening in wine. Additional sugar, yeast and chemicals are added according to the wine type and taste.
6. Bottling: After the wine has aged, it is prepared for bottling and consumption.

In addition to these steps, other modifications can be made depending on the specific wine type, such as filtration to remove any precipitate formed during the fermentation process, secondary fermentation after bottling for champagne, cooling or heating to influence the fermentation process (STE Michelle Wine Estates, 2021).

For this research project, the metal content of 2 wines from a local wine producer and 1 imported wine are determined. The local wines are Dubru Du and Kruidenwijn with an alcohol percentage of 15%, while the imported Carlo Rossi wine has an alcohol percentage of 12%. The Carlo Rossi wine is made from cabernet sauvignon grapes. The red wine is on the dryer side and is produced in the United States of America. The Dubru Du wine is produced by a local company. It is a sweet wine made from the dobrudua plant (strychnos melinoniana) that is found in the interior of Suriname (Figure 1) (Tropilab inc., n.d.). It is believed to have a libido stimulating effect and the wine is marketed towards this.

Figure 1: Dobrudua plant (Tropilab inc., n.d.)

The Kruidenwijn is a sweet wine made from various plants and spices that are believed to stimulate blood production. The production process of the wines is confidential (policy of the company), but in general, the juice is extracted from the plant and is set for fermentation and aging for approximately 1.5 years. For the Kruidenwijn the aging process is a little shorter than that of Dubru Du, because of the spices in this wine, it ages faster than the Dubru Du wine. During the process, no other chemicals apart from yeast and sugar are added to the wine. The tanks used for this process are IBC (Intermediate Bulk

Containers) tanks. The type of yeast used for these processes is specially imported from The Netherlands. (Amania, B. Personal Interview, November 30, 2021)

2.2: Metals in wine

Metals, in general, are classified into four categories:

1. Class A: metals that are essential for life, could be consumed in high amounts, e.g., iron;
2. Class B: metals that have no biological function and are not toxic at low concentration, e.g., strontium;
3. Class C: metals that are essential for life, but must be consumed in low concentrations, e.g., copper, zinc;
4. Class D: metals with no known biological function, but are toxic even at low concentrations, e.g., lead, mercury, cadmium (Durguti, Aliu, Laha, & Feka, 2020).

Metals in wine can exist in different forms such as free ions, complexes with organic acids, peptides, proteins, polyphenols, pectic polysaccharides, etc. (Dumitriu, Teodosiu, Morosanu, Jitar, & Cotea, 2019)

Metals found in wine can be:

- Macro elements: with a concentration greater than 10 ppm such as sodium (Na), potassium (K), magnesium (Mg), calcium (Ca).
- Microelements: with a concentration between 10 ppb and 10 ppm such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and lead (Pb).
- Ultra microelements: with a concentration lower than 10 ppb such as chromium (Cr), arsenic (As), cadmium (Cd) and nickel (Ni). (Voica, Dehelean, & Pamula, 2009)

Metals play a key role in the alcoholic fermentation process, such as pH and ionic balance, which regulates the cellular metabolism of yeast. The metals responsible for this are sodium, potassium, calcium and magnesium. Copper, iron, manganese and zinc are used by yeast for metalloenzyme activation. (Fabjanowicz & Płotka-Wasylka, 2021). Copper and iron at high concentration levels can alter the redox systems of the wine and form complexes with tannins and phosphates. Copper complexes are more active than iron complexes. (Stavilof & Karadjova, 2009)

Since metals can influence the chemical composition of the wine they are a crucial aspect of quality control processes of wines, hence influence the stability, taste, color and haze formation of the wine. (Ronkainen, 2016).

Metals in wine can originate from natural and anthropogenic sources such as soil, metal contamination during the process, use of pesticides and fertilizers, etc. The metal concentration can vary during the various phases of winemaking. It can either increase due to contamination or it may decrease due to yeast consumption (Durguti et al., 2020). Metals can enter the wine and accumulate during different stages of the winemaking process (Skendi, Papageorgiou, & Stefanou, 2020). Some of the possible metal contaminant sources in wines are:

- The soil where the raw material for the wine comes from, the use of pesticides, fungicides and fertilizers and surrounding environmental conditions.
- The chemicals added during the brewing process e.g. the addition of copper sulfate ($CuSO_4$) in wine to remove sulfur compounds that cause odors which increases the copper concentration.
- Type of yeast and yeast strain used during the fermentation process: the yeast used during the winemaking process can affect the sensory properties and quality of the wine. (Chambers & Pretorius, 2010)

- The process type: the steps which are taken during the winemaking process. Some steps such as distillation can remove certain impurities of wine.
- The process equipment: reaction between the metal vessels that are used during the process and its content can cause contamination.
- The bottling process, e.g., water used during the bottling process can introduce certain metals in the wine, and
- The aging and storage: metal ions such as Iron (III) can affect the stability of the wine by forming reactive species and thus cause redox reactions. Metals can also form complexes, which can affect the wine. Storage containers such as cans and bottles are also possible metal contaminant sources. (Ibanez et al., 2008)

Apart from effects on the chemical composition, metals have certain possible health effects on consumers. Some of the possible effects are toxicity in case of excessive intake. Overdose of metals can cause disturbance in ionic balance and mineral regulations, oxidative damage to cell structure, deoxyribonucleic acid (DNA) injuries, and inducement of cancerous tissue. (Formicki, Stawarz, Gren, & Muchacka, 2012). Some of the possible health effects of metal overdose are described below.

Class D metals such as lead (Pb) are toxic even at low concentrations and can cause lead poisoning. This condition can have many symptoms depending on the exposure level, varying from headache, intellectual disabilities, constipation, coma, and even death (World Health Organization, 2019). Class C metals are essential for life, but must be consumed in low concentrations. Copper and zinc are part of many important metabolic processes in the human body such as white and red blood cell formation. Increased copper concentration in blood can cause copper toxicity and lead to diarrhea, headaches and kidney failure (Eske, 2020). Zinc overdose can cause nausea, vomiting, stomach pain, diarrhea, etc. Increased zinc absorption can lead to copper deficiency because both metals compete for absorption in the small intestine (Meixner, 2018). Class A metals are essential for life. In the human body, iron is used to make hemoglobin (which transports oxygen from the lungs to all parts of the body), myoglobin and some hormones. Iron deficiency can lead to weakness, tiredness, laziness, weakened immune system, while an iron overdose can lead to stomach pain, constipation, vomiting, nausea, fainting, decreased zinc absorption, etc. Extreme Iron poisoning can cause organ failure, convulsions, coma, and even death (National Institute of Health, 2021).

The International Organization of Vine and Wine has established maximum acceptable limits of these metals in wines (International Organization of Vine and Wine, 2021) (Table 1). The determination of the metal concentration in wines was done by an AAS measurement.

Table 1: Maximum Acceptable Limits (OIV)

Metal	Maximum acceptable Limit (ppm)
Lead (Pb)	0.15
Copper (Cu)	1.0
Iron (Fe)	10
Zinc (Zn)	5

2.3: Analytical procedures

For the determination of metals in wine, several methods have been adapted in the literature. Electroanalytical methods such as differential pulse anodic stripping voltammetry (DPASV) was used in a research project done by Maciel, Souza, Silva and Dias (2019). It was found that this method could be used without sample preparation, and it yielded acceptable results with good precision and accuracy. Multielement analysis such as Inductively Coupled Plasma Spectrometry with a mass spectrometer (ICP-MS) was used by other studies. For this method, the wine samples were treated with nitric acid and hydrogen peroxide followed by microwave digestion system (Bora, et al., 2017). Plotka,

Frankowski, Simeonov, Polkowska and Namie snik (2018) used Inductively Couples Plasma Optical emission spectrophotometry (ICP-OES) and ICP-MS for the diluted samples without any pretreatment of the wine samples. Another study done by Plotka, Rutkowksa, CieVlik, Tyburcy and Namie Vnik (2017) used AAS such as Flame AAS (FAAS), Cold Vapor AAS (CVAAS), Graphite furnace AAS (GFAAS) and Atomic Emission Spectroscopy (AES) to determine trace metals in homemade fruitwines. Dragusha, Zogaj, Ramadani and Susaj (2017) used FAAS and hydride generation AAS for the determination of heavy metals in wines. The samples were preserved with nitric acid and directly nebulized. Angelova, Ivanov, Braikov and Ivanov (1999) used the FAAS method to determine metal content in wine and grapes. The wine samples were treated with nitric acid and heated untill color change.

For the determination of the metals in this research project, the FAAS method is used. This was done, because FAAS was the only available, accessible and affordable option for this project. Atomic Absorption Spectrometry (AAS) is a spectrometry technique used to trace metal species in samples at ppb - ppm concentration levels. The principle of this technique is based on the absorbance of highly monochromatic UV light, which causes the atoms of the analyzed sample to enter the excited state. The atoms absorb a portion of the light, causing a decrease in the intensity of light. The difference between light intensity before and after the absorbance of the sample is measured. The sample is burned at an extremely high temperature to atomize the sample. The atoms absorb light from the hollow cathode lamp (Figure 2) that is made from the same metal as the analyte. The hollow cathode lamp consists of an inert gas, an anode and a cathode sealed in quartz glass. The gas molecules are ionized and collide with the metal, causing the metal ions to enter the excited state. The excited ions give light that is absorbed by the analyte. The monochromator selects the wavelength that reaches the detector (Figure 3) (Loyd, n.d.). Samples can absorb light at different wavelengths and so the concentration can be determined using Lambert- Beer's Law $A= \varepsilon cl$ where A is the absorbance of the sample, ε is the molar absorptivity and l is the path length the light has travelled in the sample. The light absorbed from the sample is directly proportional to the concentration (Harris, 2010).

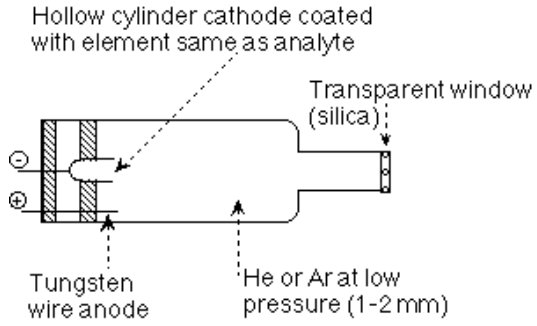

Figure 2: Hollow Cathode Lamp (Loyd, n.d.)

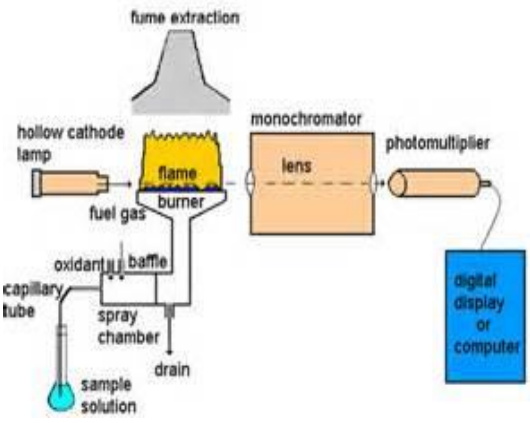

Figure 3: Overview of AAS instrumentation (Loyd, n.d.)

For this research project, the concentration of the metals is determined by two methods:

1. Using the calibration curve method: calibration standards are prepared, and the absorbance is measured. A graph is made with the concentration on the x-axis and absorbance on the y-axis. Using the linear equation of the line, the concentration of the samples can be determined.
2. Using standard addition method: four samples of each wine flavor at the beginning (juice) and at the end (wine) are made. One of the samples is spiked after extraction to determine the concentration and whether the matrix effect is present. A graph of the absorbance versus the volume of spike added is constructed. Using the equation of the graph and appropriate dilution calculations, the concentration of the sample is determined.

For quality control, one of the samples is spiked before the extraction process to determine the percentage recovery (accuracy) of the method. The following formula is used to determine the percentage recovery where C stands for the concentration:

$$\%\, recovery = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100\%$$

A calibration curve is a graphical tool used to determine the concentration of samples. The graph consists of the instrument response on the y-axis and the increasing concentration on the x-axis. A line is obtained from the data points. The line can be curved depending on the concentration, but a linear range is preferred. The calibration standards that are prepared to construct the curve are external calibration standards, meaning that the matrix of the standards and the sample is not completely the same. This could lead to matrix effects. This occurs when something in the sample other than the analyte increases or decreases the instrument signal. Since wine is a chemically complex matrix, matrix effect are most likely to occur. To combat matrix effects, standard addition method is used. Standard addition method is a technique where known small amounts of a standard are added to the sample. This causes an increase in signal. The concentration of the analyte is determined by subtracting the concentration of the standard from the total concentration. Since the standard is added in the same matrix as the analyte, matrix effects are minimized. Spiking for accuracy is also a form of standard addition to determine the accuracy of the method. The difference is that for the accuracy spike, the sample is spiked before the extraction process and goes through the same process as the unspiked sample. This can give an indication of sample loss, reactions during the extraction process or contamination during the experiment. (Harris, 2010).

Chapter 3: Materials and Methods

The materials and chemicals that were used for the practical work of this project are shown in Table 2.

Table 2: Necessities for this research project

Materials:	Chemicals:
• Standard Laboratory glassware • Analytical balance (METTLER AJ100) • Pipettes (0.5 mL, 1 mL, 2 mL, 5 mL, 10 mL) • Flame Atomic Absorption spectrophotometer (ZEEnit 700p Perkin Elmer) • Hotplate • Plastic bottles (150 mL)	• Concentrated Nitric acid (HNO_3) (Merck , 65%) • Lead nitrate ($Pb(NO_3)_2$) (Unknown brand/purity) • Iron nitrate nonahydrate ($Fe(NO_3)_3$. 9 H_2O) (Sigma Aldrich, 98%) • Copper sulfate pentahydrate ($CuSO_4$. 5 H_2O) (Unknown brand/purity) • Zinc standard 1000 ppm (Merck Centipur) • Ethanol (C_2H_5OH) (SAB, 96%) • Distilled water • Deionized water (Jong Tjien Fa N.V.) • Wine samples (Dubru Du, Kruidenwijn, Carlo Rossi, Dubru Du juice, Kruidenwijn juice)

3.1: Sample specification

The locally produced wines used in this research project are Dubru Du and Kruidenwijn. The foreign sample was Carlo Rossi Cabernet Sauvignon wine. The juices used for this research project are of Dubru Du and Kruidenwijn. All the samples were given a code. This code consists of the abbreviation of the wine or juice, the number 1 or 2 and the letters A or B.

The wines Dubru Du, Kruidenwijn and Carlo Rossi are abbreviated as DD, KW, and CR, respectively. The juices of Dubru Du and Kruidenwijn are abbreviated as DDS and KWS, respectively.

The unspiked samples were prepared in duplicate and the number 1 and 2 were assigned to these samples. For example: the unspiked duplicate samples of Dubru Du juice were coded as DDS 1 and DDS 2. Each solution was measured a couple of times, therefore A and B were added to the code (e.g. DDS 1-A and DDS 1-B (the same solution, measured 2 times)).

For the spiked samples of the standard addition method, the abbreviation SA was added behind the abbreviation of the wine or juice (e.g. DDS-SA). This solution was also measured 2 times; therefore, the standard addition spike sample of Dubru Du juice was as DDS-SA-A and DDS-SA-B. The same was done for all the other wine and juice samples.

For the spiked samples for the determination of the accuracy, the abbreviation AC was added behind the abbreviation of the wine or juice (e.g. DDS-AC). This solution was also measured 2 times; therefore, the standard addition spike sample of Dubru Du juice was as DDS-AC-A and DDS-AC-B. The same was done for all the other wine and juice samples.

3.2: Preparatory work

All the glassware used for this research project was washed with soap and tap water. After washing, the glassware was first rinsed with distilled water (3 times) and deionized water (1 time). The beakers used for the extraction process were soaked overnight in a nitric acid (1%) solution.

A nitric acid solution (1%) was made by adding concentrated nitric acid (7.70 mL, 0.19 moles) to a volumetric flask (500 mL) and diluted to the mark with deionized water.

An ethanol solution (15%) was made by adding the provided ethanol (158 mL, 2.71 moles) to a volumetric flask (1000 mL) and diluted to the mark with deionized water.

3.3: Sample preservation and extraction

The wine and juice samples (750 mL) were obtained from the company and concentrated nitric acid (7.50 mL, 0.18 moles) was added to preserve the metal ions. The samples were transferred to plastic bottles and labeled accordingly. The plastic bottles were stored at room temperature.

For the extraction process, each wine sample (50 mL) was added to a beaker and concentrated nitric acid (5 mL) was added. The beaker was covered with a watch glass and heated for 30 minutes till the remaining solution was approximately 10 mL. The solution was set to cool down to room temperature. Hereafter the solution was quantitatively transferred to a volumetric flask (100 mL) containing concentrated nitric acid (1 mL) and diluted to the mark with deionized water. This was done for all the wine samples.

A method blank was prepared by heating concentrated nitric acid (5 mL) for approximately 10 minutes. Hereafter the solution was set to cool down and quantitatively transferred to a volumetric flask (100 mL) and diluted to the mark with deionized water.

3.4: Determination of the metal concentration using the calibration curve method

For this method, a series of calibration standards of increasing concentration was prepared. The prepared calibration standards were in the linear working range of the instrument. (Perkin Elmer, 1996). The absorbances of the calibration standards and wine samples were measured. The absorbance of the calibration standards were corrected with the absorbance of the reagent blank (0 ppm calibration standard) and a graph was constructed of the corrected absorbance versus the concentration of the calibration standards. The absorbances of the wine samples was corrected with the method blank. Using the equation of the graph, the concentration of metals in the wine samples were determined.

3.4.1: Preparation of the stock solutions and stock intermediate solutions

Stock solutions (1000 ppm) for Iron, Copper, Lead and Zinc were prepared. From these solutions the stock intermediate solutions (100 ppm) solution were prepared.

The stock solutions (1000 ppm) solutions were prepared as follows:

- To prepare the lead stock solution, lead nitrate (0.3995 grams, 1.2071×10^{-3} moles) was dissolved in the 1% nitric acid solution (10 mL), next it was quantitatively transferred to a volumetric flask (250 mL) and diluted with deionized water till the mark (Perkin Elmer, 1996).
- To prepare the iron stock solution, iron nitrate nonahydrate (1.7776 grams, 4.3954×10^{-3} moles) was dissolved in the 1% nitric acid solution (10 mL), next it was quantitatively transferred to a volumetric flask (250 mL) and diluted with deionized water till the mark.
- To prepare the copper stock solution, copper sulfate pentahydrate (0.98224 grams, 3.9359×10^{-3} moles) was dissolved in the 1% nitric acid solution (10 mL), next it was quantitatively transferred to a volumetric flask (250 mL) and diluted with deionized water till the mark.
- For zinc, the 1000 ppm standard was given by Mr. Fauz Sawirjo. The zinc standard (1000 ppm) was purchased by the Central Laboratory, BOG. This was done because the zinc salt available at the Chemistry lab at the university was completely melted and had expired.

The 100 ppm solutions were made by pipetting 10 mL of the 1000 ppm solution into a volumetric flask (100 mL) containing concentrated nitric acid (1 mL) and diluting with deionized water till the mark.

3.4.2: Preparation of the calibration standards

The calibration standards were prepared from the 100 ppm solutions as shown in the tables below: Iron (Table 3), Copper (Table 4), Lead (Table 5) and Zinc (Table 6). The calibration standards were prepared in 100 mL volumetric flasks containing 1 mL concentrated nitric acid and diluted to the mark with ethanol (15%). After preparation, the solutions were immediately transferred to plastic bottles.

Table 3: Calibration standards for iron

Concentration (ppm)	Volume to take from 100 ppm (mL)
0 (reagent blank)	0
1	1
2	2
4	4
6	6

Table 4: Calibration standards for copper

Concentration (ppm)	Volume to take from 100 ppm (mL)
0 (reagent blank)	0
1	1
2	2
4	4
5	5

Table 5: Calibration standards for lead

Concentration (ppm)	Volume to take from 100 ppm (mL)
0 (reagent blank)	0
0.5	0.5
1	1
10	10
20	20

Table 6: Calibration standards for zinc

Concentration (ppm)	Volume to take from 100 ppm (mL)
0 (reagent blank)	0
0.1	0.1
0.5	0.5
1	1

3.5: Accuracy of the method

To determine the accuracy of the method, the recovery percentage was determined. The samples were spiked before the extraction process and went through the same process as the unspiked wine samples. The absorbance was measured and the concentration was determined as mentioned above. The recovery percentage was determined by the formula stated below, where C stands for concentration.

$$\%\, recovery = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100\%$$

For this part, 4 samples of Dubru Du wine (DD) were spiked before the extraction process. The spiking was done as follows:

In 4 beakers 50 mL of the wine sample and 5 mL concentrated nitric acid were added.

- To one of the beakers, 1.5 mL of the 1 ppm lead standard was added.
- To another one of the beakers, 0.5 mL of the 1000 ppm iron standard was added.
- To another one of the beakers, 2 mL of the 5 ppm copper standard was added.

- To the last beaker, 5 mL of the 1 ppm zinc standard was added.

The beakers were covered with a watch glass and heated for 30 minutes till the remaining solutions were approximately 10 mL. The solutions were set to cool down to room temperature. Hereafter the solutions were quantitatively transferred to volumetric flasks (100 mL) containing concentrated nitric acid (1 mL) and diluted to the mark with deionized water. This was repeated for the remaining samples of DDS, KW, KWS, and CR.

3.6: Determination of the concentration using the Standard Addition Method

For this method, a spiked sample is measured alongside the wine samples. Standard addition is used when there is a case of matrix effect. This occurs when something in the matrix other than the analyte causes a change in the analytical signal. The absorbances of the unspiked and the spiked samples are measured and a graph of the absorbance versus the volume of spike added is constructed. The x-intercept (y=0) gives the volume of the spike which is equivalent to the amount of analyte found in the sample. From here, with appropriate dilution calculations, the concentration of the analyte in the sample is determined.

The sample extraction was carried out as mentioned in section 3.3 till the remaining solution was approximately 15 mL. For each wine type, 6 samples were prepared. Two of the samples served as unspiked samples, whereas the rest of the samples were spiked after the extraction process, but before dilution with deionized water. For the extracted samples 10 mL was pipetted into volumetric flasks (100 mL). The spiking was done as follows:

- To one of the volumetric flasks, 1.5 mL of the 1 ppm Lead standard was added.
- To another one of the volumetric flasks, 0.5 mL of the 1000 ppm Iron standard was added.
- To another one of the volumetric flasks, 2 mL of the 5 ppm Copper standard was added.
- To the last volumetric flask, 5 mL of the 1 ppm Zinc standard was added.

All the flask contained 1 mL concentrated nitric acid and were diluted till the mark with deionized water. At the Central Lab, the absorbance of the samples was measured and corrected with the absorbance of the method blank. The standard addition graph was constructed and the calculations were done as described above.

3.7: Instrument parameters

Table 7 illustrates the instrument conditions for the ZEEnit 700p Perkin Elmer AAS instrument that was used to measure the absorbances of the calibration standards and samples.

Table 7: Instrument settings for the AAS measurement

Condition	Copper	Iron	Lead	Zinc
Wavelength	324.8 nm	248.3 nm	283.3 nm	213.9 nm
Slit Width	1.2 nm	0.2 nm	1.2 nm	0.5 nm
Light source	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode
Analytical Mode	Single beam	Single Beam	Single Beam	Single beam
Flame	Air-acetylene	Air-acetylene	Air-acetylene	Air-acetylene
Flow rate (L/h)	40	45	45	70
Nebulizer rate (mL/m)	5.0	5.0	5.0	5.0

3.8: Statistical analysis and method validation

The unspiked wine samples were prepared in duplicate. The data are expressed as mean ± the standard deviation. For constructing the graphs and doing the calculations, Microsoft Excel was used. Q test was used for outliers.

Method validation gives an indication of how well the chosen method is suited for this research project. The following criteria are used for the method validation of this project.

Limit of detection (LOD): the concentration level to which a method can reliably give a signal statistically different from the background signal (noise). At 95% confidence level, the LOD is determined by the concentration that produces a signal that is 3 times the standard deviation of the blank. The LOD is calculated as follows: $\frac{3s}{slope\ of\ calibration\ curve}$ where s is the standard deviation of the blank.

Limit of quantification (LOQ): the smallest amount that can be accurately measured. At 95% confidence level, LOQ is determined by $\frac{10s}{slope\ of\ calibration\ curve}$ where s is the standard deviation of the blank.

Sensitivity: the ability of the instrument to distinguish minor changes in analyte concentration. This was determined from the slope of the calibration curve. The steeper the calibration curve, the more sensitive the method is.

Linearity: measure of how well the calibration curve follows a straight line. The value is given by the square of the correlation coefficient (R^2). If the value of R^2 lies close to 1, the calibration curve is linear.

Precision: how well the obtained values are relative to each other. It is determined by the standard deviation, the relative standard deviation, the coefficient of variation, the variance and the standard error of the samples using the equations given below. Precision was determined for samples that were measured 5 times.

Standard deviation = $S_x = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$, where $\bar{\boldsymbol{x}}$ is the mean (average) of a number of samples (n) and $\boldsymbol{x_i}$ is a specific data point.

Relative standard deviation = $\frac{s}{\bar{x}}$, where $\bar{\boldsymbol{x}}$ is the mean (average) and s is the standard deviation.

Coefficient of variation: $\frac{s}{\bar{x}} \times 100\%$, where $\bar{\boldsymbol{x}}$ is the mean (average) and s is the standard deviation.

Variance: s^2, where s is the standard deviation of the data set

Standard error of the mean: $S_m = \frac{s}{\sqrt{N}}$, where s is the standard deviation of the data set and N is the number of data points (Harris, 2010; Skoog, 2007)

Chapter 4: Results

4.1: Determination of the metal concentration using the calibration curve method

4.1.1: Determination of the concentration of iron

Table 8 shows the concentration of the wine samples obtained by the calibration curve (Figure 4). The absorbances and corrected absorbances are given in appendix A (Table 24).

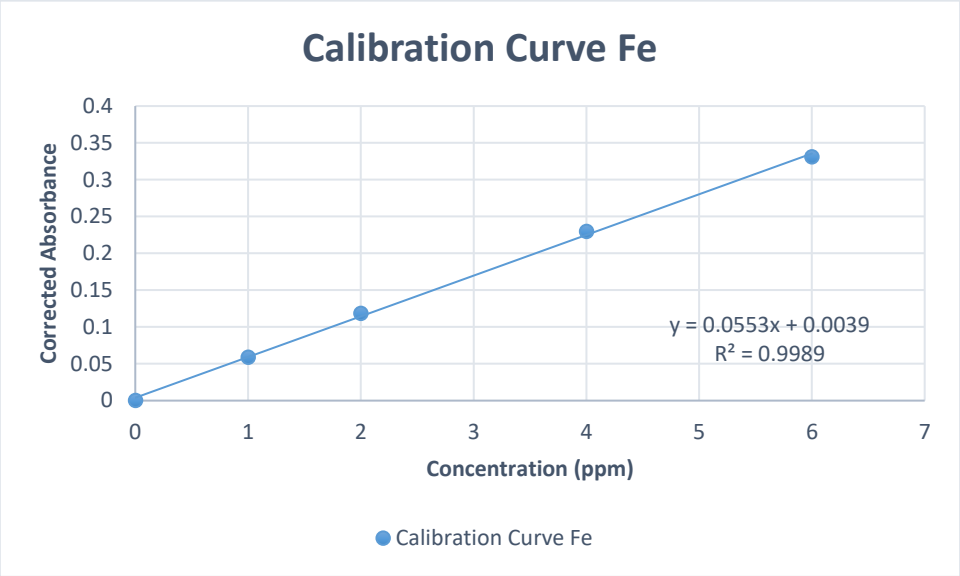

Figure 4: Calibration curve of iron standards

The concentration of the samples was determined as follows:

Equation of the graph => $y = 0.0553x + 0.0039$

Concentration of CR 1-A => $0.05152 = 0.0553x + 0.0039 => x = \frac{0.05152 - 0.0039}{0.0553} = 0.86$ ppm

The exact concentration was determined by expressing all the data points for CR 1 (A and B) as mean ± the standard deviation. The average concentration was determined by the average of the data points of CR 1 and CR 2 ± the appropriate uncertainty. The final concentration was determined by the average concentration x the dilution factor*

*The dilution factor for the samples was determined to be $\frac{757.5\ mL}{50\ mL} = 15.15$

A bottle of wine sample (750 mL) was preserved with 7.5 mL concentrated nitric acid. That makes the final volume of the wine sample 757.5 mL. For the extraction of the wine/juice samples, 50 mL was taken from the bottle for each sample.

The concentration per bottle was determined by the final concentration x dilution factor*

Dilution factor** was determined as $\frac{757.5\ mL}{750\ mL} = 1.01$

**A second dilution factor was calculated to determine the concentration in a bottle of wine/juice that had a volume of 750 mL.

In table 8, the concentration of the iron samples for the calibration method is presented. In the second column of table 8, the concentration is determined from the calibration curve (see appendix A for sample calculation). In the third column the exact concentration is presented which is determined by the mean ± the standard deviation, for e.g. sample CR 1-A and CR 1-B. The average concentration (fourth

column) is determined by taking the average of the exact concentrations for the duplicate samples (1 and 2). The last column gives the concentration per bottle which is calculated as mentioned above.

Table 8: Concentrations of iron samples

Sample	Concentration (ppm)	Exact Concentration (ppm)	Average concentration (ppm)	Final Concentration (ppm)	Concentration/bottle (ppm)
CR-1 A	0.86	0.86 ± 0.01	0.73 ± 0.05	11.06 ±0.76	11.17 ± 0.77
CR-1 B	0.87				
CR-2 A	0.50	0.59 ± 0.05			
CR-2 B	0.58				
CR-2 B	0.64				
CR-2 A	0.61				
CR-2A	0.62				
DDS-1 A	0.78	0.78 ±0.01	0.76 ± 0.01	11.51 ± 0.21	11.63 ± 0.22
DDS-1 B	0.78				
DDS-2 A	0.72	0.73 ± 0.01			
DDS-2 B	0.75				
DDS-2 A	0.73				
KWS-1 A	1.22	1.23 ± 0.01	1.23 ± 0.02	18.63 ± 0.30	18.82 ± 0.30
KWS-1 B	1.23				
KWS-2 A	1.25	1.23 ± 0.02			
KWS-2 B	1.22				
KW-1 A	1.86	1.85 ± 0.01	1.67 ± 0.02	25.3 ± 0.30	25.55 ± 0.30
KW-1 B	1.84				
KW-2 A	1.47	1.48 ± 0.02			
KW-2 B	1.50				
DD-1 A	1.99	2.04 ± 0.08	2.22 ± 0.08	33.63 ± 1.21	33.97 ± 1.22
DD-1 B	2.10				
DD-2 A	2.39	2.40 ± 0.02			
DD-2 B	2.41				

4.1.2: Determination of the concentration of copper

Table 9 shows the concentration of the copper wine samples determined by the calibration curve (Figure 5). The concentration is determined the same way as described in section 4.1.1. The absorbances and corrected absorbances are given in appendix A (Table 26). When constructing the calibration curve for copper the 1 ppm standard and 2 ppm standard were discarded because the values were too high, causing negative results for the copper concentration. When these were discarded, positive concentrations were obtained.

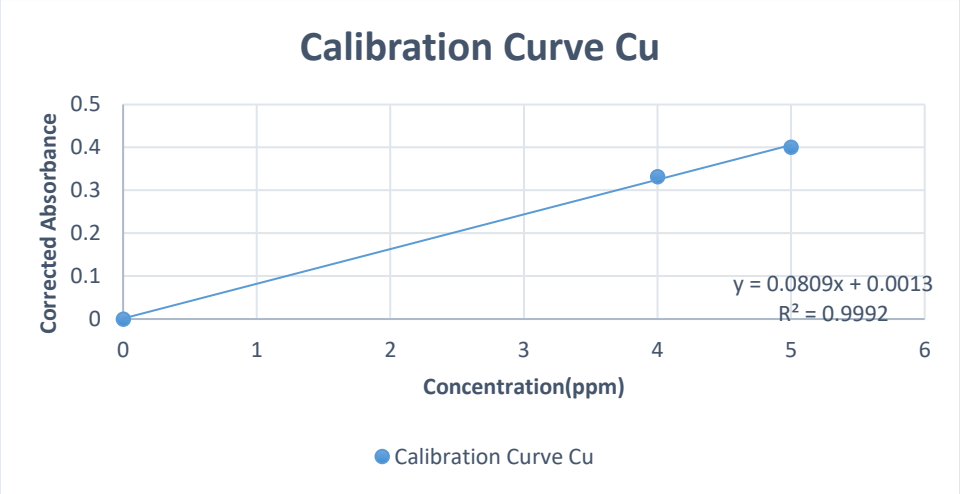

Figure 5: Calibration curve of copper standards

Table 9:Concentrations of copper samples

Sample name	Concentration (ppm)	Exact Concentration (ppm)	Average Concentration (ppm)	Final Concentration (ppm)	Concentration/bottle (ppm)
CR 1-A	0.14	0.14 ± 0.01	0.11 ± 0.01	1.67 ± 0.03	1.69 ± 0.03
CR 1-B	0.14				
CR 2-A	0.08	0.08 ± 0.01			
CR 2-B	0.08				
DDS 1-A	0.09	0.09 ± 0.01	0.09 ± 0.01	1.36 ± 0.01	1.37 ± 0.01
DDS 1-B	0.09				
DDS 2-A	0.09	0.08 ± 0.01			
DDS 2-B	0.08				
KWS 1 -A	0.07	0.07 ± 0.01	0.08 ± 0.01	1.21 ± 0.09	1.22 ± 0.09
KWS 1-B	0.07				
KWS 2-A	0.08	0.08 ± 0.01			
KWS 2-B	0.08				
KW 1-A	0.01	0.01 ± 0.01	0.01 ± 0.01	0.15 ± 0.05	0.15 ± 0.05
KW 1-B	0.01				
KW 2-A	0.01	0.01 ± 0.01			
KW 2-B	0.01				
DD1-A	0.00	Below LOD	-	-	
DD1-B	0.00				
DD2-A	-0.02	Below LOD			
DD2-B	-0.02				

4.1.3: Determination of the concentration of lead

Figure 6 shows the calibration curve for lead. The exact concentration of the lead standards and absorbances and corrected absorbances are given in appendix A (Table 27 and 28)

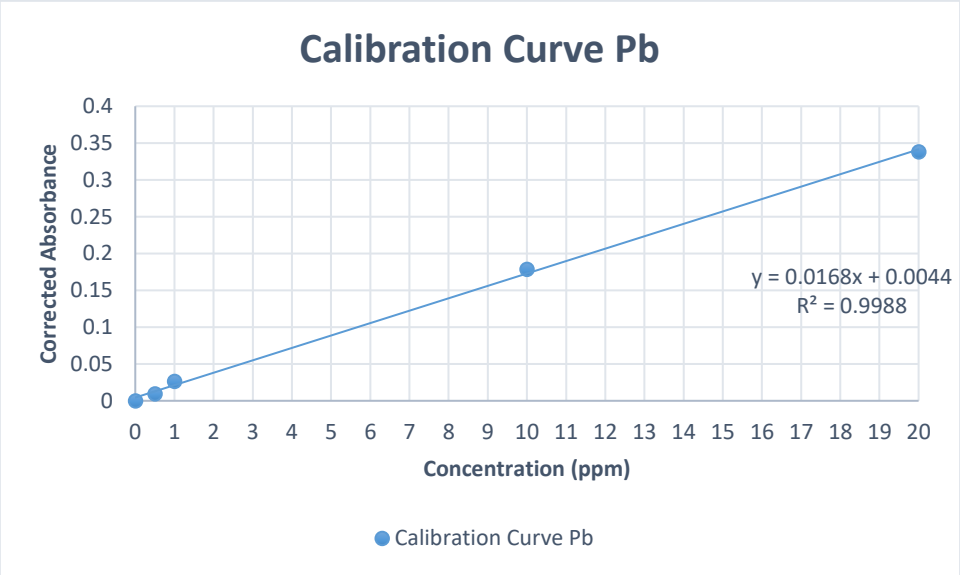

Figure 6: Calibration curve for lead standards

The concentration of lead could not be determined as all the obtained absorbances of the wine samples were negative. This indicates that the concentration of lead in the samples were below the detection limit of the instrument.

4.1.4: Determination of the concentration of zinc

As stated earlier, the 1000 ppm zinc standard was obtained from BOG. Table 10 shows the concentration of the zinc wine samples determined by the calibration curve (Figure 7). The concentration is determined the same way as described in section 4.1.1. The absorbances and corrected absorbances are given in appendix A (Table 30).

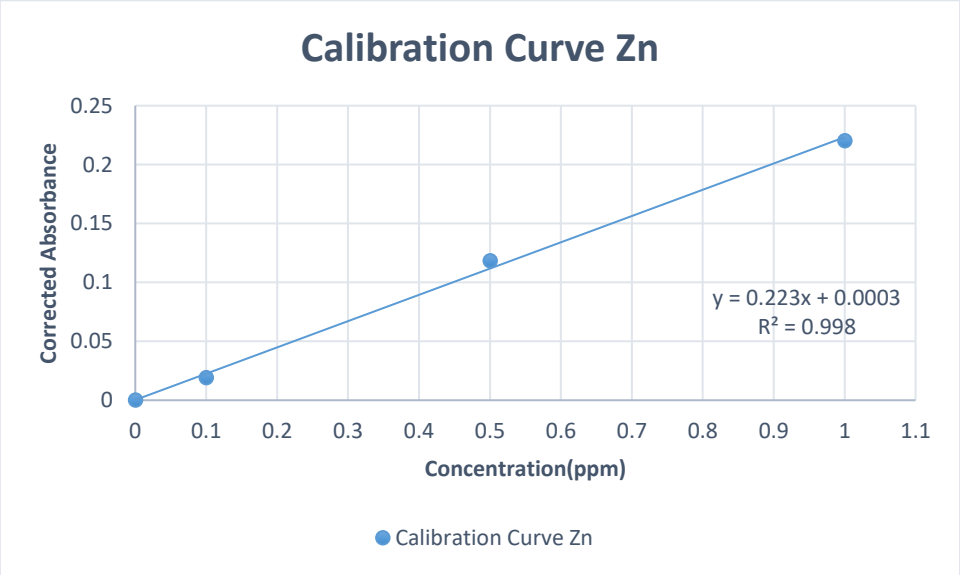

Figure 7: Calibration curve for zinc standards

Table 10: Concentration of the zinc samples

Sample	Concentration (ppm)	Exact Concentration (ppm)	Average Concentration (ppm)	Final Concentration (ppm)	Concentration/bottle (ppm)
DD1-A	0.39	0.39 ± 0.01	0.36 ± 0.01	5.50 ± 0.15	5.55 ± 0.15
DD1-B	0.39				
DD2-A	0.33	0.32 ± 0.01			
DD2-B	0.32				
KW1-A	0.52	0.51 ± 0.02	0.43 ± 0.02	6.5 ± 0.3	6.6 ±0.3
KW1-B	0.50				
KW2 -A	0.35	0.34 ± 0.01			
KW2-B	0.34				
CR1-A	0.10	0.09 ± 0.06	0.09 ± 0.06	1.36 ± 0.91	1.37 ± 0.92
CR1-B	0.04				
CR1-A	0.19				
CR1-A	0.08				
CR2-A	0.05				
DDS1-A	-0.24	Below LOD	-	-	-
DDS1-B	-0.24				
DDS 2-A	-0.09				
DDS2 -B	-0.12				
KWS1-A	-0.11	Below LOD	-	-	-
KWS1-B	-0.12				
KWS2-A	-0.11				
KWS 2-B	-0.15				

4.2: Determination of the concentration of the metals using the standard addition method

4.2.1: Iron determination

Table 11 shows the concentration of iron in the wine samples as determined by the standard addition method. Figure 8 shows the standard addition curve for the CR sample. Using MS Excel the x-intercept was determined and using appropriate dilution calculations the final concentration and concentration per bottle was determined. The standard addition graphs and sample calculation are given in appendix B. (Figure 12,13,14,15)

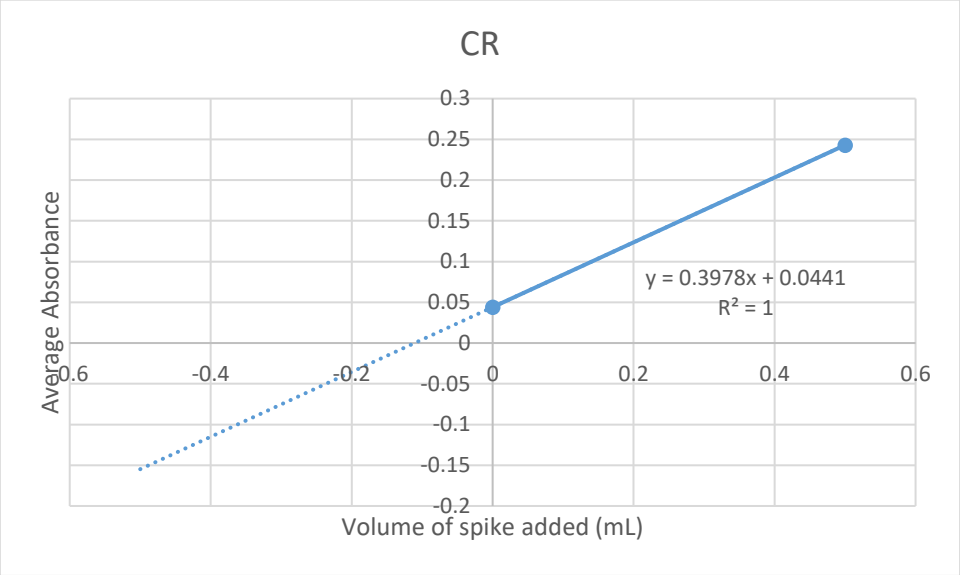

Figure 8: Standard addition graph of iron for CR sample

Table 11: Data for the standard addition calculation for iron

Sample Name	X - intercept	Final concentration (ppm)	Concentration/ bottle (ppm)
CR	-0.1108	50.336	50.839 ± 0.115
DDS	-0.0843	38.292	38.675 ± 0.020
KWS	-0.1999	90.887	91.786 ± 0.344
KW	-0.3098	140.759	142.166 ± 0.505
DD	-0.4438	201.616	203.632 ± 0.138

4.2.2: Copper determination

Table 12 shows the concentration of copper in the wine samples as determined by the standard addition method. Using MS Excel the x-intercept was determined and using appropriate dilution calculations the final concentration and concentration per bottle was determined. The standard addition graphs and sample calculation are given in appendix B. (Figure 16,17,18,19,20)

Table 12: Data for the standard addition calculation for copper

Sample Name	X - intercept	Final concentration (ppm)	Concentration/ bottle (ppm)
CR	-0.4853	1.102	1.113 ± 0.011
DDS	-1.1743	2.643	2.669 ± 0.016
KWS	-1.1230	2.554	2.580 ± 0.014
KW	-0.2806	0.636	0.643 ± 0.014

DD	-0.1004	0.228	0.231 ± 0.006

4.2.3: Lead

The concentration for lead using the standard addition method could not be determined, because all absorbance values were negative.

4.2.4: Zinc determination

Table 13 shows the concentration of zinc in the wine samples as determined by the standard addition method. Using MS Excel the x-intercept was determined and using appropriate dilution calculations the final concentration and concentration per bottle was determined. The standard addition graphs and sample calculation are given in appendix B. (Figure 21,22,23). The data of samples DDS and KWS were discarded because of the negative absorbance values obtained when conducting the AAS measurement.

Table 13: Data for the standard addition calculation for zinc

Sample Name	X – intercept	Final concentration (ppm)	Concentration/ bottle (ppm)
CR	7.6287	4.772	4.820 ± 2.525
KW	53.1894	24.429	24.674 ± 1.400
DD	-28.2389	13.069	13.200 ± 1.774

4.3: Method Validation

4.3.1: Limit of Detection

The limit of detection gives the concentration level to which a method can reliably give a signal statistically different from the background signal (noise). The LOD (Table 14) was determined by $\frac{3\,S\,of\ blank}{Slope}$ at 95% confidence level.

For iron the S of the 0 ppm standard was 0.000282

$$\frac{3 \times 0.000282}{0.0553} = 0.015\ ppm$$

In the same way the LOD for the other metals was determined. For the determination of the LOD, the 0 ppm calibration standard was used instead of the method blank, because the method blank was not measured enough times to provide the necessary data, while the 0 ppm standard did provide the data.

Table 14: Determination of LOD of the metals

Metal	SD of 0 ppm standard	LOD (ppm)
Iron	0.000282	0.015
Copper	0.000329	0.012
Lead	0.000166	0.03
Zinc	0.02247	0.302

4.3.2: Limit of Quantification

The LOQ gives the smallest amount that can be accurately measured. The LOQ (Table 15) is determined by $\frac{10\,S\,blank}{Slope}$ at 95% confidence level.

For iron the S of the 0 ppm standard was 0.000282

$$\frac{10 \times 0.000282}{0.0553} = 0.05\ ppm$$

In the same way the LOQ for the other metals was determined. For the determination of the LOQ, the 0 ppm calibration standard was used instead of the method blank, because the method blank was not measured enough times to provide the necessary data, while the 0 ppm standard did provide the data.

Table 15: Determination of LOQ of the metals

Metal	SD of 0 ppm standard	LOQ (ppm)
Iron	0.000282	0.05
Copper	0.000329	0.04
Lead	0.000166	0.01
Zinc	0.02247	1.00

4.3.3: Sensitivity

The sensitivity of an instrument can be given by the slope of the calibration curve. The steeper the curve, the more sensitive the instrument is. Table 16 shows the slopes of all the 4 calibration curves. The slopes are reported with the uncertainty. The uncertainty was determined with MS Excel. The greater the slope, the steeper the curve. This means that the instrument was most sensitive to Zinc, because Zinc has the greatest slope and least sensitive to Lead, because lead has the smallest slope.

Table 16: Slope of the calibration curves

Metal	Slope of Calibration curve
Iron	0.0553 ± 0.0010
Copper	0.0809 ± 0.0020
Lead	0.0168 ± 0.0003
Zinc	0.2223 ± 0.0070

4.3.4: Linearity

Table 17 shows the linearity of the calibration curves. All of the obtained values lie close 1 to. This means that the calibration curves are linear.

Table 17: Correlation coefficient of the calibration curves

Metal	R^2
Iron	0.999
Copper	0.999
Lead	0.999
Zinc	0.998

4.3.5: Precision

Precision of the data is shown in table 18. For iron and copper the precision was measured from the concentration of the CR-2 sample and KWS -AC sample, respectively. For lead and zinc, the precision

was measured from the concentration of the 10 ppm calibration standard and the 0.5 ppm calibration standard respectively. The precision criteria is calculated as mentioned in section 3.8

Table 18: Precision criteria

Metal	Mean	Standard Deviation	RSD %	Variance	Standard error of mean
Iron	0.59	0.05	9.5	0.003	0.025
Copper	0.20	0.01	2.4	2.334E-05	0.002
Lead	9.99	0.24	2.4	0.058	0.108
Zinc	0.54	0.07	13.2	0.005	0.032

4.3.6: Determination of percentage recovery of iron

Table 19 shows the accuracy calculation for Iron.

The concentration of added spike for Iron was 5 ppm.

The calculation is done using the formula:

$$\%\, recovery = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100\%$$

The percentage recovery for sample CR-AC-A is done as follows: $\frac{3.82-0.67}{5} \times 100\% = 63\%$

The percentage recovery for the other samples and other metals is determined in the same way.

Table 19: Data for the accuracy calculation for iron

Sample	Absorbance	Corrected Absorbance	Concentration spiked sample (ppm)	Concentration unspiked sample (ppm)	Recovery %
CR-AC A	0.2287	0.2149	3.82	0.67	63
CR-AC B	0.2283	0.2145	3.81		63
DDS- AC A	0.2261	0.2123	3.77	0.75	60
DDS-AC B	0.2259	0.2121	3.77		60
KWS- AC A	0.2464	0.2326	4.14	1.23	58
KWS- AC B	0.2501	0.2363	4.20		60
KW-AC A	0.2355	0.2217	3.94	1.67	45
KW- AC B	0.2329	0.2191	3.89		45
DD-AC A	0.2175	0.2037	3.61	2.22	28
DD-AC B	0.216	0.2022	3.59		27

4.3.7: Determination of percentage recovery of copper

Table 20 shows the percentage recovery for the copper samples. The percentage recovery is determined in the same way as described in section 4.3.6. The concentration of spike added to the copper samples was 0.1 ppm.

Table 20: Data for the accuracy calculation for copper

Sample	Absorbance	Corrected Absorbance	Concentration (ppm)	Concentration sample (ppm)	Recovery %
CR AC-A	0.03212	0.01107	0.12	0.11	13
CR AC-B	0.03172	0.01067	0.12		
DDS AC- A	0.03651	0.01546	0.17	0.09	88
DDS AC-B	0.03725	0.0162	0.18		97
KWS AC-A	0.03926	0.01821	0.21	0.07	127
KWS AC-B	0.03873	0.01768	0.20		
KWS AC-B	0.03843	0.01738	0.20		
KWS AC-B	0.03841	0.01736	0.20		
KWS AC- A	0.03829	0.01724	0.20		
KW AC- A	0.1133	0.09225	1.12	0.01	1114
KW AC-B	0.1128	0.09175	1.12		1114
DD AC-A	0.0304	0.00935	0.10	-0.01	109
DD AC-B	0.02936	0.00831	0.09		96

4.3.8: Determination of percentage recovery for lead

The percentage recovery for lead could not be determined, because all the absorbance values were negative.

4.3.9: Determination of percentage recovery for zinc

Table 21 shows the percentage recovery for the zinc samples. The percentage recovery is determined in the same way as described in section 4.3.6. The concentration of spike added to the zinc samples was 0.05 ppm.

Table 21: Data for the accuracy calculation for zinc

Sample	Absorbance	Corrected Absorbance	Concentration (ppm)	Concentration sample (ppm)	Recovery %
DD AC-A	0.2326	0.0718	0.32	0.36	-72
DD AC-B	0.2237	0.0629	0.28		-151
DD AC-A	0.2201	0.0593	0.26		-186
KW AC-A	0.2768	0.116	0.52	0.43	184
KW-AC-B	0.284	0.1232	0.55		247
KW AC-A	0.284	0.1232	0.55		247
CR AC-A	0.1831	0.0223	0.10	0.09	15
CR AC-B	0.1764	0.0156	0.07		-45
CR AC-B	0.1687	0.0079	0.03		-114
CR AC-A	0.1695	0.0087	0.04		-107

Chapter 5: Discussions

Table 22 summarizes the results found based on the two methods used:

Table 22:Summary of the concentration of the wine and juice samples

Sample	Calibration curve method				Standard Addition method			
	Fe (ppm)	Cu (ppm)	Lead (ppm)	Zinc (ppm)	Fe (ppm)	Cu (ppm)	Lead (ppm)	Zinc (ppm)
CR	11.17 ± 0.77	1.69 ± 0.03	-	1.37 ± 0.92	50.839 ± 0.115	1.113 ± 0.011	-	4.820 ± 2.525
KW	25.55 ± 0.30	0.15 ± 0.05	-	6.6 ± 0.3	142.166 ± 0.505	0.643 ± 0.014	-	24.674 ± 1.400
KWS	18.82 ± 0.30	1.22 ± 0.09	-	-	91.786 ± 0.344	2.580 ± 0.014	-	-
DD	33.97 ± 1.22	-	-	5.55 ± 0.15	203.632 ± 0.138	0.231 ± 0.006	-	13.200 ± 1.774
DDS	11.63 ± 0.22	1.37 ± 0.01	-	-	38.675 ± 0.020	2.669 ± 0.016	-	-

Iron

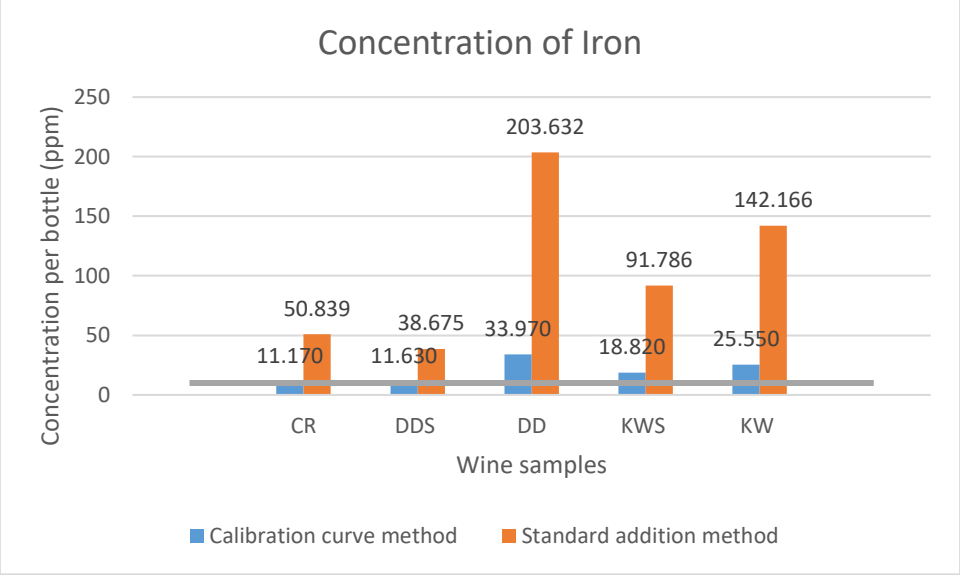

Figure 9: Concentration of iron in the wine and juice samples

From the calibration curve method it was found that all the wine and juice samples exceeded the O.I.V. limit for iron (10 ppm). The same result was found for these samples from the standard addition method (Figure 9)

The samples before the wine making process (KWS and DDS) had a lower concentration of iron than the samples after the wine making process. This could indicate that during the winemaking process the wines are contaminated with iron. The winemaking process is confidential, so it is not known in which stadium the contamination occurs. High levels of iron could occur through contamination from the water used during the process. High levels of iron (greater than 10 ppm) can alter redox systems in the wine, creating instabilities and haze formation. (Pyrzynska, 2007).

The iron concentration in KWS samples was greater than in DDS samples. This could indicate that the ingredients that are used to make Kruidenwijn have a higher iron content than the ingredients that are used to make Dubru Du wine. However, at the end of the winemaking process, Dubru Du wine has a greater iron concentration than Kruidenwijn. The difference in percentage of iron between wine and juice of Dubru Du is 43.24% and 32.95% for the standard addition method and the calibration curve method, respectively. The reason for this could be that the Dubru Du wine is longer in the winemaking process than the Kruidenwijn, so there are more possibilities for contamination for the Dubru Du wine.

The increase in iron concentration between Dubru Du juice samples and Dubru Du wine samples is greater than that of Kruidenwijn juice and wine samples. For the calibration method the percent increase between juice and wine is 192.1% and 35.76% for the Dubru Du samples and the Kruidenwijn samples, respectively. For the standard addition method, the percent increase between juice and wine is 426.53% and 54.89% for Dubru Du samples and Kruidenwijn samples. This could indicate that there is more iron contamination during the Dubru Du wine making process than the Kruidenwijn making process.

When Dubru Du wine and Kruidenwijn are compared to the foreign Carlo Rossi wine, the iron concentration of Dubru Du and Kruidenwijn is greater than the Carlo Rossi wine. The iron concentration of Carlo Rossi wine determined by the calibration method slightly exceeds the O.I.V. limit for iron (11.7%), while that of the locally produced wines significantly exceeds the limit, 239.3 % and 155.5% for Dubru Du wine and Kruidenwijn, respectively. For the standard addition method, all the wine samples exceeded the O.I.V. limit for iron by more than 400%.

Copper

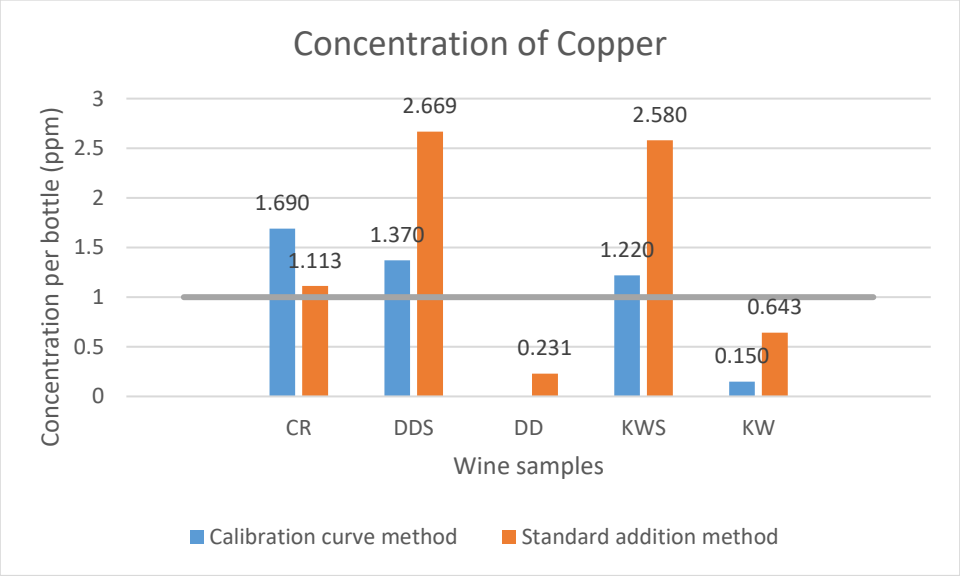

Figure 10: Concentration of copper in the wine and juice samples

For copper, the values for concentration per bottle of Dubru Du and Kruidenwijn wine samples were lower than the OIV limit (1.0 ppm). For the calibration curve method, copper was not detected in Dubru Du wine samples. For the Carlo Rossi wine and both juices (calibration curve method and standard addition method) the concentration of copper slightly exceeded the limit (Figure 10).

The samples before the wine making process (KWS and DDS) were significantly higher in copper concentration than the samples after the winemaking process. This could indicate that copper is reacting with something and that process decreases the copper concentration in the wine. According to a study done by Durguti et al. (2020), yeast present in the wine is shown to consume copper and/or precipitate along with the metals during the fermentation process which can cause a decrease in copper concentration of the wines when compared to their respective juices.

The copper concentration in DDS samples was greater than in KWS samples. The percent increase in concentration between DDS and KWS samples was 12.29% and 4.61% for the calibration curve method and the standard addition method, respectively. This could indicate that the ingredients that are used to make Dubru Du wine have a higher copper content than the ingredients that are used to make Kruidenwijn wine. However, at the end of the winemaking process the copper content of Dubru Du wine is lower than that of Kruidenwijn. This could be due to the fact that Dubru Du wine is longer in the winemaking process, so the yeast can consume more of the copper in this wine than the Kruidenwijn.

When Dubru Du wine and Kruidenwijn are compared to the foreign Carlo Rossi wine, the copper concentration of Dubru Du and Kruidenwijn is lower than the Carlo Rossi wine for the calibration method and the standard addition method. The locally produces wines do not exceed the O.I.V. limit for copper, while the foreign wine does slightly exceed the limit.

Zinc

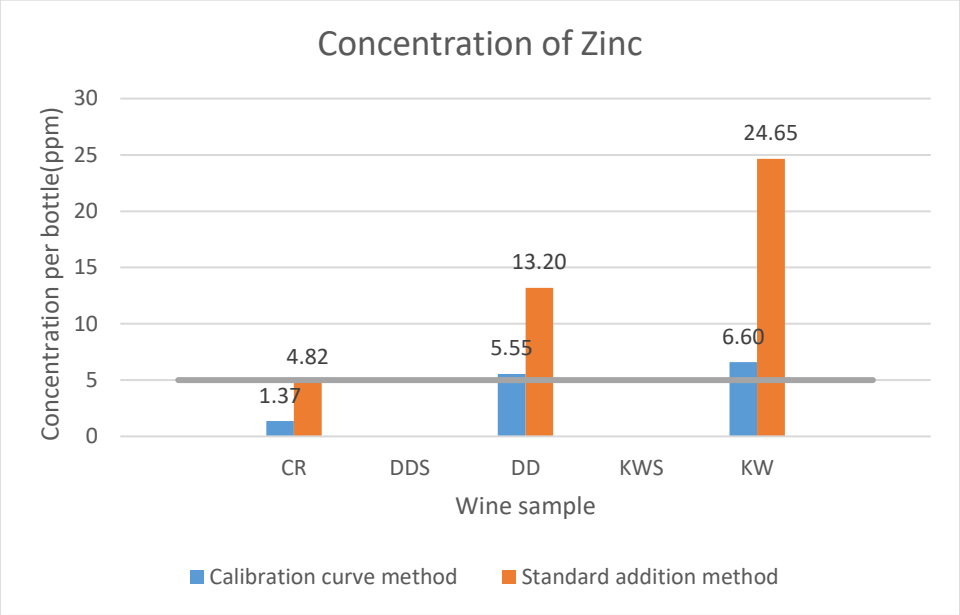

Figure 11: Concentration of zinc in the wine and juice samples

From the calibration curve method it was found that the locally produced wines exceeded the O.I.V. limit for zinc (5 ppm). For the standard addition method it was found that the Dubru Du wine and Kruidenwijn exceed the O.I.V. limit. Zinc was only detected in the end product (KW and DD) for both methods (Figure 11). This could indicate that during the winemaking process there is zinc contamination. This could be because the ingredients used during the winemaking process were contaminated with zinc containing pesticides. (Durguti, Aliu, Laha, & Feka, 2020). The obtained results could also be due to the fact that other metals could have interfered with the signal, causing an increase in the signal. For the standard addition method for CR and KW samples an error occurred. The x-intercept was positive, whereas normally it should be negative. This occurs when the concentration of the spiked sample is lower than the concentration of the unspiked sample. That could happen during the experiment when an error could be made when preparing the solutions.

When Dubru Du wine and Kruidenwijn are compared to the foreign Carlo Rossi wine, the zinc concentration of Dubru Du and Kruidenwijn is greater than the Carlo Rossi wine for the calibration curve method and the standard addition method. The Carlo Rossi wine does not exceed the O.I.V. limit for zinc, while the concentration of locally produced wines do exceed the limit.

Lead

Lead was not detected in the samples. All the samples gave negative absorbances. From this it can be concluded that the concentration of lead in the wine samples is below the detection limit (0.03 ppm), which is lower than the limit stated by O.I.V. (0.15 ppm). The spiked samples also gave negative absorbances. This was due to the fact that the lead samples were spiked with a concentration that was below the detection limit, so it could not create a significant increase in the signal to be detected.

Standard Addition method versus calibration curve method

The results obtained from the calibration method and the standard addition method are significantly different. This could be due to the fact that there was matrix effect present in the sample. Something was interfering with the analyte causing an increase in the signal. Matrix effect is verified, because the slopes of the calibration curves and the standard addition curves are different from each other. For the standard addition method, the matrix of the sample and the analyte is the same, so a more accurate concentration is determined. A 2 point standard addition curve was used for this project. This could limit the accuracy of the method, since a greater number of datapoints could provide more accurate data.

Method Validation

The instrument used for this project was most sensitive for zinc and least sensitive to lead. This is because zinc has the greatest slope out of the 4 metals, while lead has the smallest slope (Table 16). Zinc was also determined to be the metal with the highest limit of detection, while copper had the lowest limit of detection (Table 14). The correlation coefficient for all the 4 metals were found to be remarkably close to 1. This indicates that the curves were linear. The precision of the method indicates that copper and lead had produced data with less scatter than zinc and iron. For accuracy of the method the results were different for each of the wine samples. For iron the accuracy was between 27% and 63%. For copper the accuracy was between 13% and 1114%. For zinc the accuracy was between -186% and 247%. The varying range of percentage recovery for the metals could indicate experimental errors made during the practical part, sample loss, reactions during the heating process in some cases and contamination in other cases. The low and negative values for the accuracy could be because the metals were reacting with the matrix, therefore for the calibration curve method lower values for the concentration were obtained.

Chapter 6: Conclusions and Recommendations

The aim for this research project was to determine the concentration of iron, copper, lead and zinc in locally produced wines, their respective juices and a foreign wine to see whether or not the metal concentrations were below the limits stated by the O.I.V. The concentration of iron in the samples determined by the calibration curve method was found to be ranging from 11.2 ± 0.8 ppm to 34.0 ±1.2 ppm. While, for the standard addition method, the concentration of iron was found to be range from 38.7 ± 0.1 ppm to 203 ± 0.138 ppm. The concentration of copper in the samples determined by the calibration curve method was found to be ranging from 0.150 ± 0.05 ppm to 1.69 ± 0.03 ppm. While for the standard addition method, the copper concentration was ranging from 0.231 ± 0.006 ppm to 2.67 ± 0.02 ppm. The concentration of zinc in the samples determined by the calibration curve method was found to be ranging from 1.37 ± 0.92 ppm to 6.60 ± 0.3 ppm. While for the standard addition method, the concentration range was 4.82 ± 2.53 ppm to 24.6 ± 1.4 ppm. For lead, all the absorbance values were negative, thus meaning that the concentration of lead was below the limit of detection of the instrument (0.03 ppm). Iron and zinc in the locally produced wines exceeded the International Organization of Wine and Vine (O.I.V.) limit, while copper and lead were below the limits. When the wines were compared to their respective juices, it was found that the juices had a lower iron and zinc concentration than the wines. But, for copper, it was found that the concentration in the juices was higher than in the corresponding wines. When compared to a foreign wine, the levels of iron, copper and zinc found in the locally produced wines were overall higher than in the foreign wine.

Recommendations

It is recommended that a more sensitive and faster instrument such as Inductively Coupled Plasma Mass Spectrometer (ICP-MS) is used for wine analysis, because of the more accurate, fast results and a lower LOD for the ICP method. It is also recommended that a wine pretreatment step such as microwave digestion is used to have another matrix than this method which could be easier to analyze because less interferences are present. Adjusting the calibration range to a smaller one could also improve the accuracy of the results. Spiking with higher concentrations than this method could also provide more accurate results, because a significant increase in analyte concentration is created. For the accuracy of the method it is also recommended to do a 4 point standard addition curve, instead of a 2 point standard addition curve. Bentonite fining can reduce the concentrations of some metals such as Copper and Zinc (Durguti, et al, 2020). Chemical techniques such as ion exchange, precipitation, chelating agents, the use of a polymer with the trade name divergan HM were shown to decrease copper and iron concentration. Another method to remove metals in wine is: first, raising the pH with sodium bicarbonate ($NaHCO_3$) or calcium carbonate ($CaCO_3$), then add tannins or tannic acid and let react for a few days, hereafter add gelatin and bentonite to the mixture and stir, decant and filter. This method is shown to decrease iron, copper and zinc concentrations. (Ibanez et al., 2008). Since it is not known in which stadium of the winemaking process the metal contamination occurs, it is recommended that samples during various stages of the winemaking process should be taken and the metal concentration should be determined to see if and how the metal content varies. Doing so could possibly trace the metal contamination more precisely.

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Appendix A: Calibration curve method

Preparation of 1000 ppm solutions, 100 ppm solutions and calibration standards:

To prepare the 1000 ppm Iron solution, 1.7757 grams of $Fe(NO_3)_3.9H_2O$ was dissolved in 250 mL 1% nitric acid. The exact concentration of this solution was determined as follows:

$$\frac{1.7757\ grams\ Fe(NO3)3\ .9H2O}{403.99\frac{g}{moles}} = 4.3954\ \times 10^{-3}\ moles$$

$Fe(NO_3)_3\ .\ 9H_2O$: Fe^{3+} => 1:1 => $4.3954\ \times 10^{-3}\ moles$ $Fe(NO_3)_3.9H_2O$ Δ $4.3954\ \times 10^{-3}\ moles$ Fe^{3+}

$4.3954\ \times 10^{-3}\ moles$ Fe^{3+} = $4.3954\ \times 10^{-3}\ moles\ \times 55.845\frac{g}{mole} = 0.2455\ grams = 245.5\ mg$ Fe^{3+}

Concentration of Fe^{3+} stock solution = $\frac{245.5\ mg}{0.250\ L} = 981.85\ ppm$

Actual concentration of the 100 ppm intermediate solution => $981.85\ ppm \times \left(\frac{10}{100}\right) = 98.185\ ppm$

Table 23:Determination of the exact concentrations the iron calibration standards

Calibration standard	Actual concentration
0 ppm standard	$98.185\ ppm\ \times \left(\frac{0}{100}\right) = 0\ ppm$
1 ppm standard	$98.185\ ppm\ \times \left(\frac{1}{100}\right) = 0.98\ ppm$
2 ppm standard	$98.185\ ppm\ \times \left(\frac{2}{100}\right) = 1.96\ ppm$
4 ppm standard	$98.185\ ppm\ \times \left(\frac{4}{100}\right) = 3.93\ ppm$
6 ppm standard	$98.185\ ppm\ \times \left(\frac{6}{100}\right) = 5.89\ ppm$

Table 24: Absorbances and corrected absorbances for iron samples

Concentration Calibration standard	Absorbance	Corrected Absorbance
0	0.002873	0
1	0.06167	0.058797
2	0.1213	0.118427
4	0.2327	0.229827
6	0.3339	0.331027
Sample	Absorbance	Corrected Absorbance
Method Blank	0.0138	0
CR-1 A	0.06532	0.05152
CR-1 B	0.06558	0.05178
CR-2 A	0.04514	0.03134
CR-2 B	0.04978	0.03598
CR-2 B	0.05301	0.03921
CR-2 A	0.05135	0.03755
CR-2A	0.05206	0.03826
DDS-1 A	0.06075	0.04695
DDS-1 B	0.06066	0.04686
DDS-2 A	0.05754	0.04374

DDS-2 B	0.05903	0.04523
DDS-2 A	0.05797	0.04417
KWS-1 A	0.085	0.0712
KWS-1 B	0.08589	0.07209
KWS-2 A	0.08677	0.07297
KWS-2 B	0.08501	0.07121
KW-1 A	0.1203	0.1065
KW-1 B	0.1193	0.1055
KW-2 A	0.09919	0.08539
KW-2 B	0.1004	0.0866
DD-1 A	0.1276	0.1138
DD-1 B	0.1336	0.1198
DD-2 A	0.1498	0.136
DD-2 B	0.1511	0.1373

Determination of concentration of samples using the calibration method:

First the absorbance of the samples obtained from the AAS measurement is corrected by the absorbance of the method blank

Sample calculation CR-1A: $Corrected\ Absorbance = 0.06532 - 0.0138 = 0.05152$

Using this corrected absorbance and the equation of the calibration curve of iron, the concentration of the CR-1 A is determined as follows:

Equation of the graph => $y = 0.0553x + 0.0039$

Concentration of CR 1-A => $0.05152 = 0.0553x + 0.0039 => x = \frac{0.05152-0.0039}{0.0553} = 0.86$ ppm

The exact concentration was determined by expressing all the data points for CR 1 (A and B) as mean ± the standard deviation (see appendix C for sample calculation for mean and standard deviation) The average concentration was determined by the average of the data points of CR 1 and CR 2 ± the appropriate uncertainty. The uncertainty for the average concentration was determined by $\sqrt{(error\ CR\ 1)^2 + (error\ CR2)^2}$ => $\sqrt{(0.01)^2 + (0.05)^2} = 0.05$

The final concentration was determined by the average concentration x the dilution factor*

Sample calculation for final concentration Fe of CR sample = 0.73 ± 0.05 ppm x 15.15 = 11.06 ppm

*The dilution factor for the samples was determined to be $\frac{757.5\ mL}{50\ mL} = 15.15$. A bottle of wine sample (750 mL) was preserved with 7.5 mL concentrated nitric acid. That makes the final volume of the wine sample 757.5 mL. For the extraction of the wine/juice samples, 50 mL was taken from the bottle for each sample.

The uncertainty value for the final concentration was determined as follows:

First the relative uncertainty was determined by $\frac{0.05}{0.73} \times 100\% = 6.85\ \%$

Then the relative uncertainty was converted to absolute uncertainty by $6.85\ \% \times 11.06\ ppm = 0.76$

The final concentration of Fe in CR sample was determined to be 11.06 ± 0.76 ppm

The concentration per bottle was determined by the final concentration x dilution factor**

Dilution factor** was determined as $\frac{757.5\ mL}{750\ mL} = 1.01$

**A second dilution factor was calculated to determine the concentration in a bottle of wine/juice that had a volume of 750 mL

For the concentration per bottle of Fe in CR sample => 11.06 ± 0.76 ppm x 1.01 = 11.17

The uncertainty value for the concentration per bottle was determined as follows:

First the relative uncertainty was determined by $\frac{0.76}{11.06} \times 100\% = 6.87\ \%$

Then the relative uncertainty was converted to absolute uncertainty by $6.87\ \% \times 11.17\ ppm = 0.77$

The concentration per bottle of Fe in CR sample was determined to be 11.17 ± 0.77 ppm

The same way the concentration of the other iron, copper and zinc samples were determined.

To prepare the 1000 ppm Copper solution, 0.98275 grams of $CuSO_4.5\ H_2O$ was dissolved in 250 mL 1% nitric acid. The exact concentration of this solution was determined as follows:

$$\frac{0.98275\ grams\ \text{CuSO4. 5 H2O}}{249.685\frac{g}{moles}} = 3.9359\ \times 10^{-3}\ moles$$

$CuSO_4.\ 5\ H_2O$: Cu^{2+} => 1:1 => $3.9359 \times 10^{-3}\ moles$ $CuSO_4.5\ H_2O$ Δ $3.9359 \times 10^{-3}\ moles$ Cu^{2+}

$3.9359\ \times 10^{-3}\ moles\ Cu^{+} = 3.9359\ \times 10^{-3}\ moles\ \times 63.55\frac{g}{mole} = 0.2501\ grams = 250.1\ mg$ Cu^{2+}

Concentration of Cu^{2+} stock solution = $\frac{250.1\ mg}{0.250\ L} = 1000.40\ ppm$

Actual concentration of the 100 ppm intermediate solution => $1000.40\ ppm \times \left(\frac{10}{100}\right) = 100.04\ ppm$

Table 25: Determination of the exact concentrations the copper calibration standards

Calibration standard	Actual concentration
0 ppm standard	$100.04\ ppm\ \times \left(\frac{0}{100}\right) = 0\ ppm$
1 ppm standard	$100.04\ ppm\ \times \left(\frac{1}{100}\right) = 1.00\ ppm$
2 ppm standard	$100.04\ ppm\ \times \left(\frac{2}{100}\right) = 2.00\ ppm$
4 ppm standard	$100.04\ ppm\ \times \left(\frac{4}{100}\right) = 4.00\ ppm$
5 ppm standard	$100.04\ ppm\ \times \left(\frac{5}{100}\right) = 5.00\ ppm$

Table 26: Absorbances and corrected absorbances for copper samples

Concentration	Absorbance	Corrected Absorbance
0	0.02239	0
4	0.3538	0.33141
5	0.4228	0.40041
Sample name	Absorbance	Corrected Absorbance
Method Blank	0.02105	0
CR 1-A	0.03355	0.0125

CR 1-B	0.03334	0.01229
CR 2-A	0.02858	0.00753
CR 2-B	0.02882	0.00777
DDS 1-A	0.02958	0.00853
DDS 1-B	0.02954	0.00849
DDS 2-A	0.02925	0.0082
DDS 2-B	0.02922	0.00817
KWS 1 -A	0.02836	0.00731
KWS 1-B	0.02763	0.00658
KWS 2-A	0.02857	0.00752
KWS 2-B	0.02881	0.00776
KW 1-A	0.02337	0.00232
KW 1-B	0.02338	0.00233
KW 2-A	0.02295	0.0019
KW 2-B	0.02334	0.00229
DD1-A	0.02206	0.00101
DD1-B	0.02199	0.00094
DD2-A	0.02114	9E-05
DD2-B	0.02112	7E-05

To prepare the 1000 ppm Lead solution, 0.39990 grams of $Pb(NO_3)_2$ was dissolved in 250 mL 1% nitric acid. The exact concentration of this solution was determined as follows:

$$\frac{0.39990\ grams\ \text{Pb(NO3)2}}{331.29\frac{g}{moles}} = 1.2071 \times 10^{-3}\ moles$$

$Pb(NO_3)_2$: Pb^{2+} => 1:1 => $1.2071 \times 10^{-3}\ moles$ $Pb(NO_3)_2$ Δ $1.2071 \times 10^{-3}\ moles$ Pb^{2+}

$1.2071 \times 10^{-3}\ moles$ Pb^{2+} = $1.2071 \times 10^{-3}\ moles \times 207.2\frac{g}{mole} = 0.2501\ grams = 250.1\ mg$ Pb^{2+}

Concentration of Pb^{2+} stock solution = $\frac{250.1\ mg}{0.250\ L} = 1000.44\ ppm$

Actual concentration of the 100 ppm intermediate solution => $1000.44\ ppm \times \left(\frac{10}{100}\right) = 100.044\ ppm$

Table 27: Determination of the exact concentrations the lead calibration standards

Calibration standard	Actual concentration
0 ppm standard	$100.044ppm \times \left(\frac{0}{100}\right) = 0\ ppm$
0.5 ppm standard	$100.044\ ppm \times \left(\frac{0.5}{100}\right) = 0.5\ ppm$
1 ppm standard	$100.044\ ppm \times \left(\frac{1}{100}\right) = 1.0ppm$
10 ppm standard	$100.044\ ppm \times \left(\frac{10}{100}\right) = 10.0\ ppm$
20 ppm standard	$100.044\ ppm \times \left(\frac{20}{100}\right) = 20.0\ ppm$

Table 28: Absorbances and corrected absorbances for lead samples

Concentration	Absorbance	Corrected Absorbance
0	-0.00026	0
0.5	0.009107	0.009367
1	0.02611	0.02637
10	0.1785	0.17876
20	0.338	0.33826
Sample name	Absorbance	Corrected Absorbance
Method Blank	-0.0103	0
DD 1-A	-0.0102	1E-04
DD1-B	-0.0108	-0.0005
DD2-A	-0.0082	0.0021
DD2-B	-0.0087	0.0016
KW1-A	-0.0124	-0.0021
KW1-B	-0.0126	-0.0023
KW2-A	-0.0143	-0.004
KW2-B	-0.0146	-0.0043
CR 1-A	-0.0162	-0.0059
CR1-B	-0.0167	-0.0064
CR2-A	-0.0171	-0.0068
CR2-B	-0.0172	-0.0069
DDS1-A	-0.0174	-0.0071
DDS1-B	-0.0178	-0.0075
DDS2-A	-0.018	-0.0077
DDS2-B	-0.018	-0.0077
KWS1 -A	-0.0196	-0.0093
KWS1-B	-0.0197	-0.0094
KWS2-A	-0.0192	-0.0089
KWS2-B	-0.0191	-0.0088

For the zinc standards, the calibration standards are determined as follows:

Table 29: Determination of the exact concentrations the zinc calibration standards

Calibration standard	Actual concentration
0 ppm standard	$100\ ppm\ \times \left(\frac{0}{100}\right) = 0\ ppm$
0.1 ppm standard	$100\ ppm\ \times \left(\frac{0.1}{100}\right) = 0.1\ ppm$
0.5 ppm standard	$100\ ppm\ \times \left(\frac{0.5}{100}\right) = 0.5\ ppm$
1 ppm standard	$100\ ppm\ \times \left(\frac{1}{100}\right) = 1\ ppm$

Table 30: Absorbances and corrected absorbances for zinc samples

Concentration	Absorbance	Corrected Absorbance

0	0.1771	0
0.1	0.1963	0.0192
0.5	0.2954	0.1183
1	0.3974	0.2203
Sample name	Absorbance	Corrected Absorbance
Method Blank	0.1608	0
DD1-A	0.2474	0.0866
DD1-B	0.249	0.0882
DD2-A	0.2344	0.0736
DD2-B	0.2315	0.0707
KW1-A	0.2774	0.1166
KW1-B	0.2718	0.111
KW2 -A	0.2383	0.0775
KW2-B	0.2376	0.0768
CR1-A	0.1827	0.0219
CR1-B	0.1701	0.0093
CR1-A	0.2028	0.042
CR1-A	0.1789	0.0181
CR2-A	0.1728	0.012
DDS1-A	0.1075	-0.0533
DDS1-B	0.107	-0.0538
DDS 2-A	0.1414	-0.0194
DDS2 -B	0.1344	-0.0264
KWS1-A	0.1366	-0.0242
KWS1-B	0.1339	-0.0269
KWS2-A	0.1362	-0.0246
KWS 2-B	0.1265	-0.0343

Appendix B: Standard addition method

Table 31: Data for the standard addition samples

	Iron		Copper		Zinc	
Sample name	Absorbance	Corrected Absorbance	Absorbance	Corrected Absorbance	Absorbance	Corrected Absorbance
CR-SA A	0.2572	0.2434	0.05504	0.03399	0.1728	0.012
CR-SA B	0.2563	0.2425	0.05445	0.0334	0.1508	-0.01
DDS-SA A	0.3299	0.3161	0.04369	0.02264	0.1396	-0.0212
DDS-SA B	0.3302	0.3164	0.04354	0.02249	0.1311	-0.0297
KWS- SA A	0.2661	0.2523	0.04126	0.02021	0.1518	-0.009
KWS- SA B	0.2647	0.2509	0.0414	0.02035	0.146	-0.0148
KW- SA A	0.2653	0.2515	0.03876	0.01771	0.2468	0.086
KW-SA B	0.2642	0.2504	0.039	0.01795	0.2478	0.087
DD-SA A	0.2834	0.2696	0.03108	0.01003	0.2566	0.0958
DD-SA B	0.2832	0.2694	0.03158	0.01053	0.2528	0.092

For the standard addition method, the concentration of the samples was determined as follows:

The sample calculation for the determination of the concentration of CR is as follows:

To spike the sample 0.5 mL of the 1000 ppm standard was added to a volumetric flask (100 mL) containing 10 mL of the wine sample.

Concentration of spike added = $\frac{0.5\ mL \times 1000\ ppm}{100} = 5\ ppm$

The X-intercept for CR was determined to be -0.1108. This was determined using the Intercept function of MS Excel. The intercept means that 0.11 mL of the 1000 ppm Iron standard correspondents to 10 mL of the wine sample

Number of mg Fe in 0.11 mL of 1000 ppm standard was determined as follows: $\frac{0.11}{1000}\ L \times 1000\ \frac{mg}{L} = 0.11\ mg$

Standard addition was done with 10 mL of 15 mL extract

Mass of Fe present in 10 mL extract is 0.11 mg, therefore mass of Fe present in 15 mL is $0.11\ mg \times 1.5 = 0.165\ mg$.

Similarly, mass of Fe present in 50 mL unextracted sample is 0.165 mg

Concentration of Fe in 50 mL unextracted sample is $\frac{0.165\ mg}{0.05\ L} = 3.3\ ppm$

Concentration of Fe after first dilution is $3.3\ ppm\ \times 15.15 = 50.0\ ppm$

Dilution factor was determined as $\frac{757.5\ mL**}{50\ mL} = 15.15$

**A bottle of wine sample (750 mL) was preserved with 7.5 mL concentrated nitric acid. That makes the final volume of the wine sample 757.5 mL.

The concentration per bottle Carlo Rossi was determined by: $50.0\ ppm \times 1.01 = 50.5\ ppm$

Dilution factor* was determined as $\frac{757.5\ mL}{750\ mL} = 1.01$

*A second dilution factor calculated to determine the concentration in a bottle of wine/juice that had a volume of 750 mL

To determine the uncertainty values associated with the concentrations determined by the standard addition method some additional calculations were made in Microsoft Excel. The absorbances of the samples CR-SA-A and CR-SA-B versus the volume of spike added were plotted individually in a graph and the x-intercept was determined (Table 32). The concentration per bottle was determined as mentioned above. The standard error of the mean was determined to obtain the uncertainty values.

Table 32: Data for the determination of uncertainties

Sample	Corrected Absorbance	x-intercept	Concentration/ bottle (ppm)
CR-SA-A	0.2434	-0.1105	50.724
CR-SA-B	0.2425	-0.1110	50.954

Mean was determined by $\frac{50.724+50.954}{2} = 50.839$

Standard deviation was determined by $s = \sqrt{\frac{(50.724-50.839)^2+(50.954-50.839)^2}{2-1}} = 0.163$

Standard error of the mean was determined by $s_m = \frac{0.163}{\sqrt{2}} = 0.115$

Exact concentration of Fe per Carlo Rossi bottle is 50.839±0.115 ppm

In the same way, the concentration of the other iron, copper and zinc samples was determined.

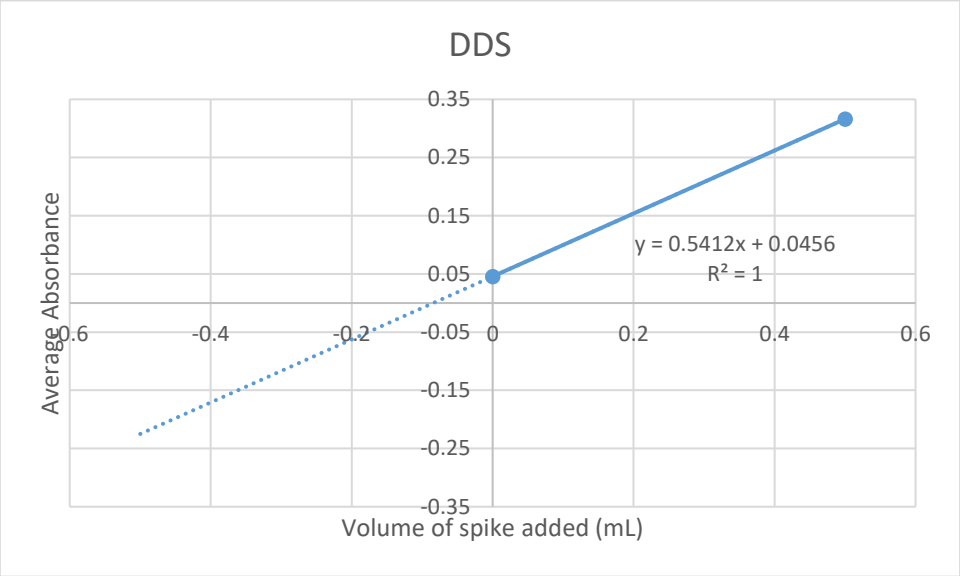

Figure 12:Standard addition curve for iron of DDS sample

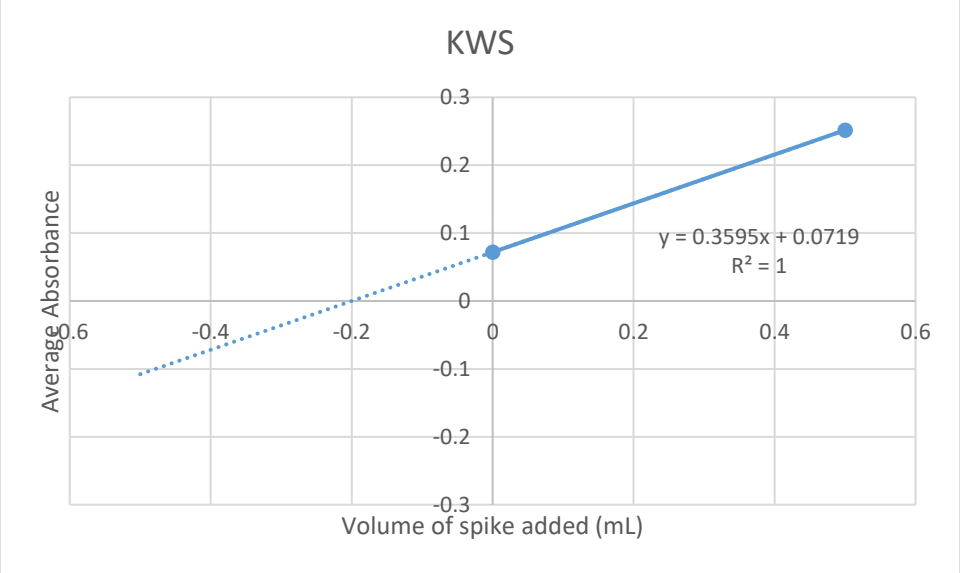

Figure 13: Standard addition curve for iron of KWS sample

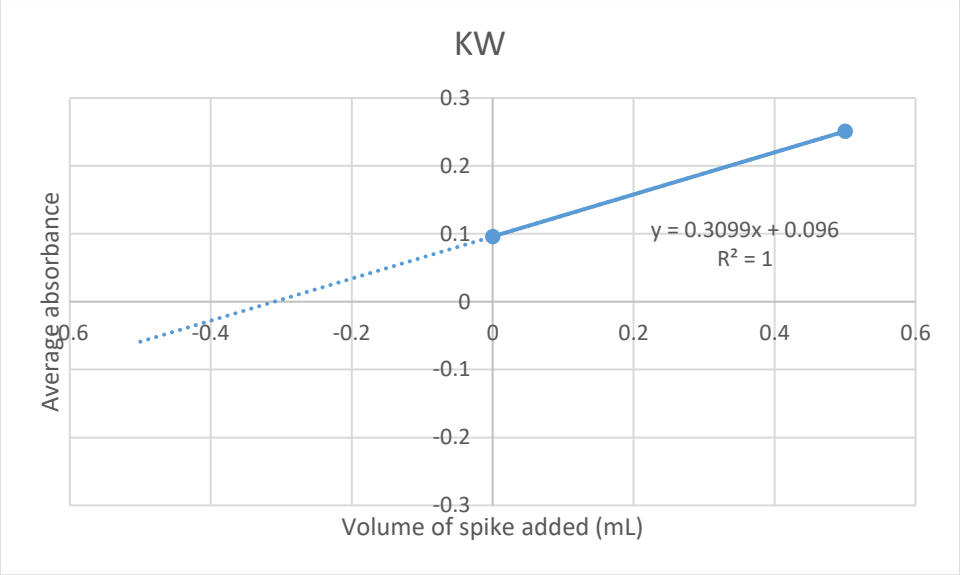

Figure 14:Standard addition curve for iron of KW sample

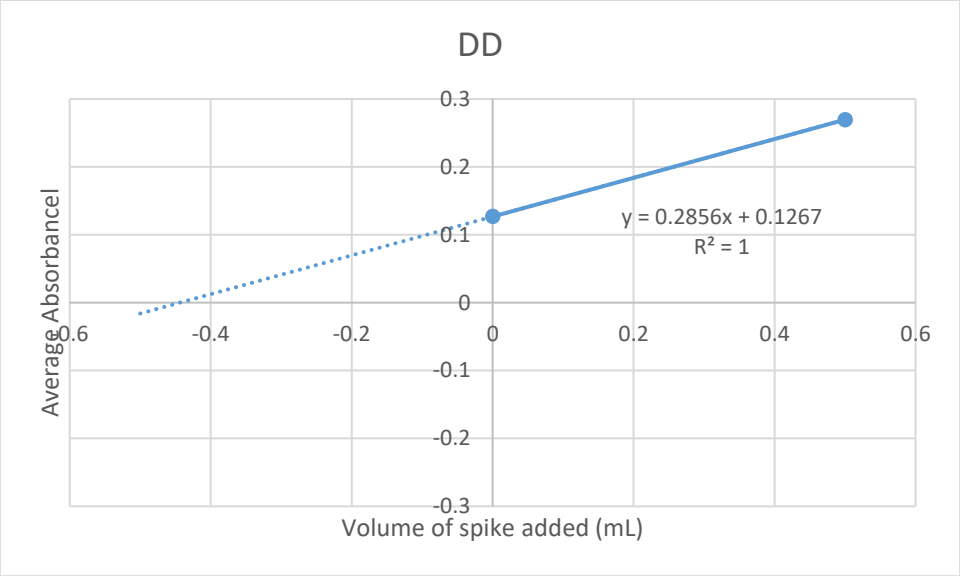

Figure 15:Standard addition curve for iron of DD sample

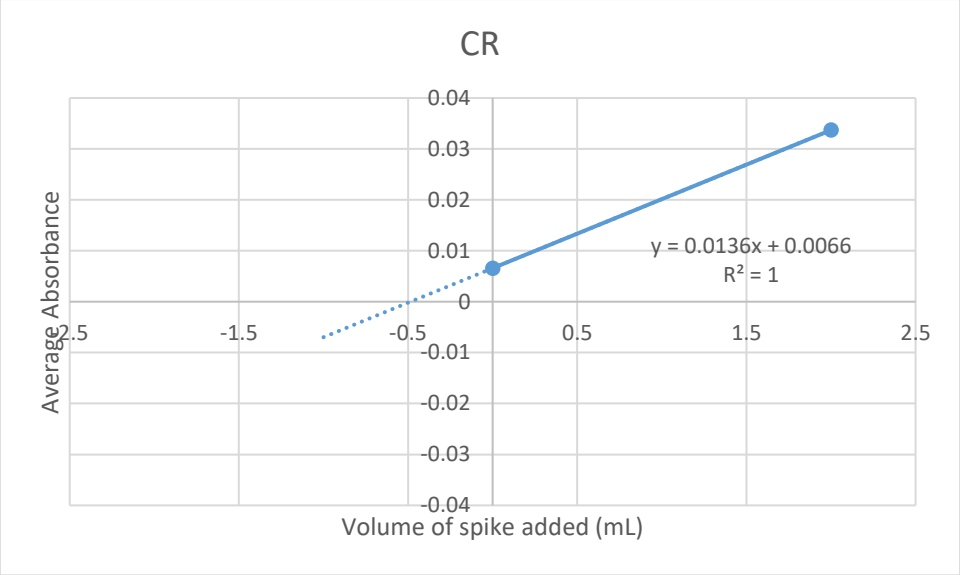

Figure 16: Standard addition curve for copper of CR sample

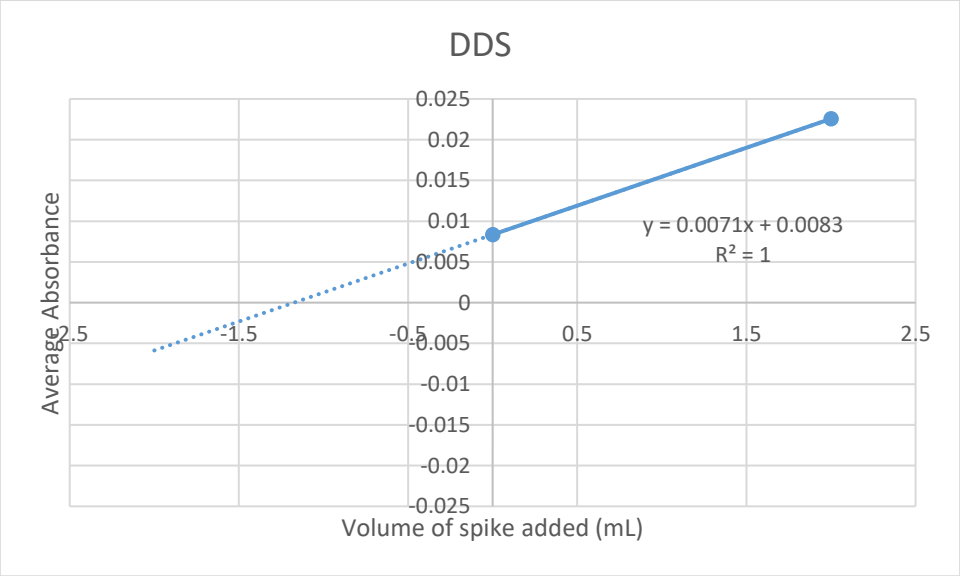

Figure 17: Standard addition curve for copper of DDS sample

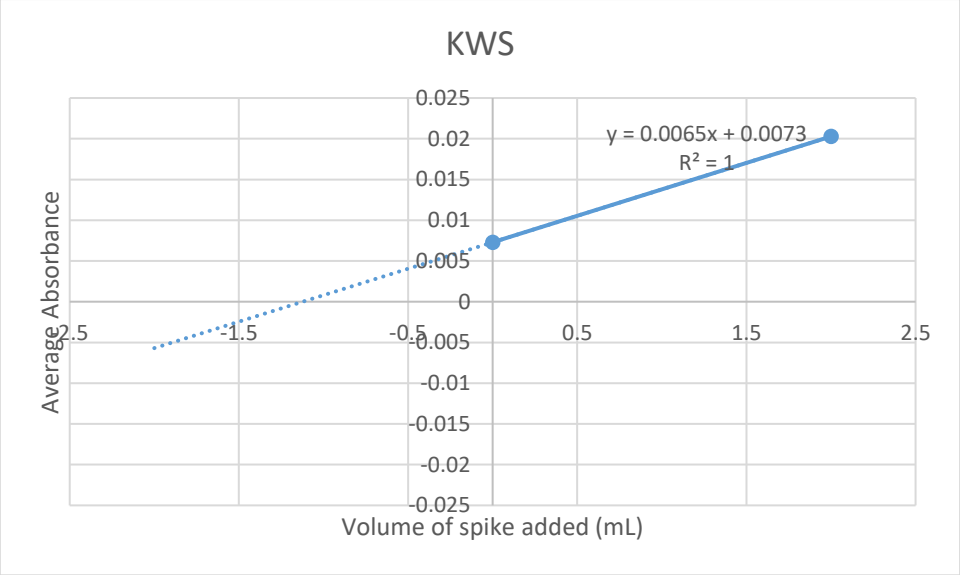

Figure 18: Standard addition curve for copper of KWS sample

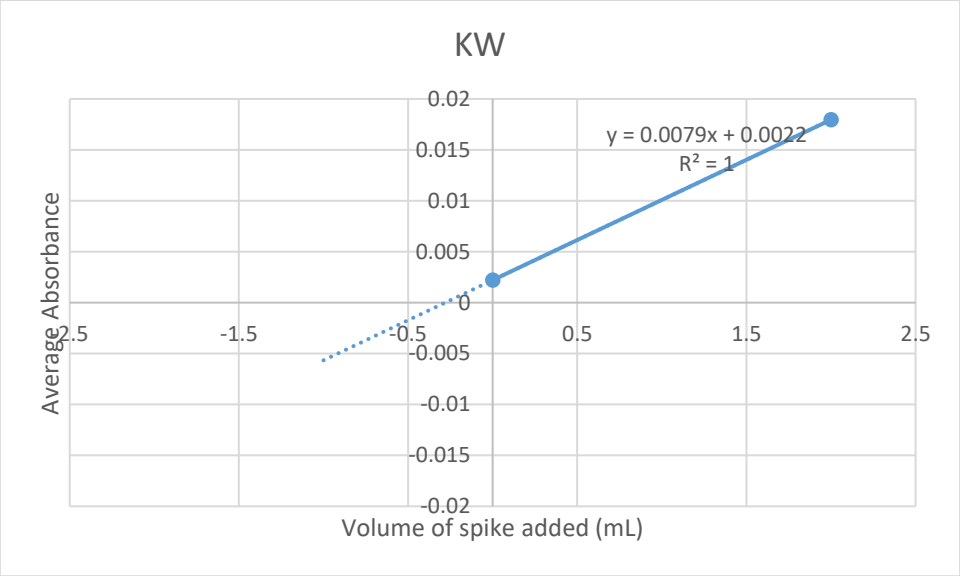

Figure 19: Standard addition curve for copper of KW sample

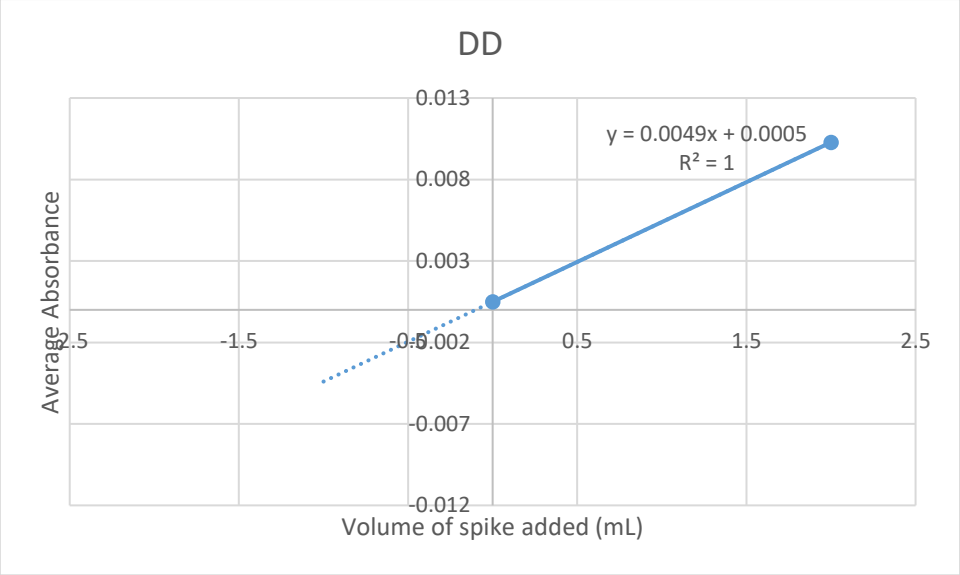

Figure 20: Standard addition curve for copper of DD sample

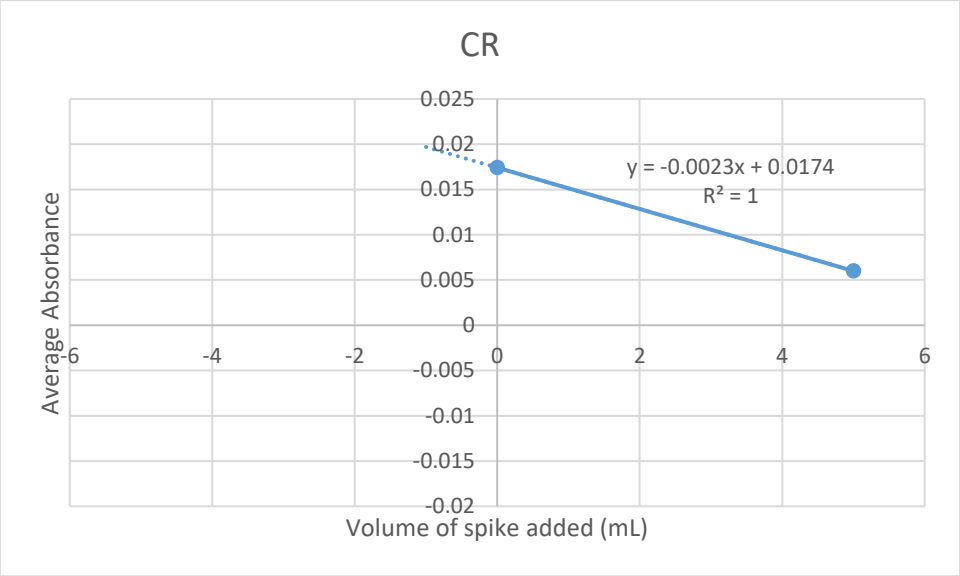

Figure 21: Standard addition curve for zinc of CR sample

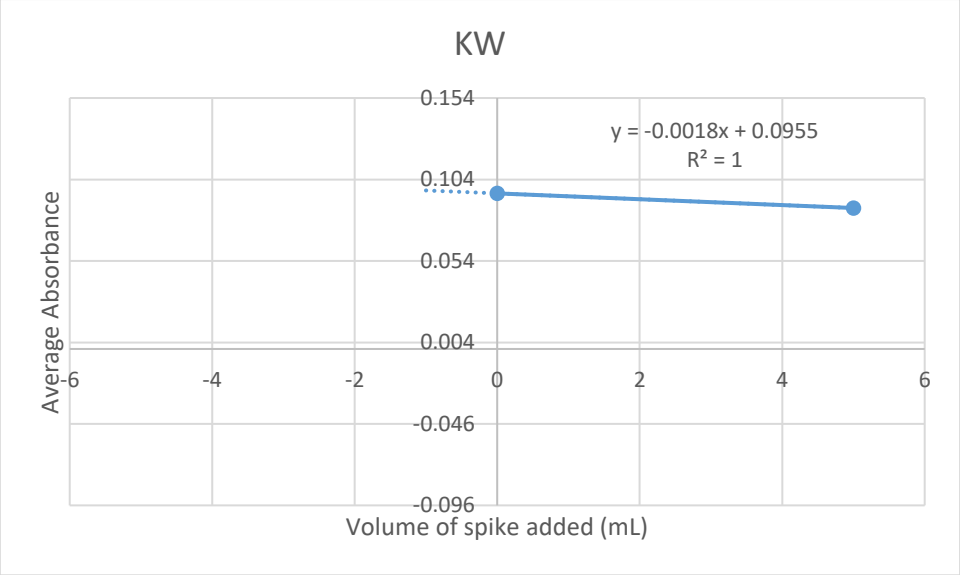

Figure 22: Standard addition curve for zinc of KW sample

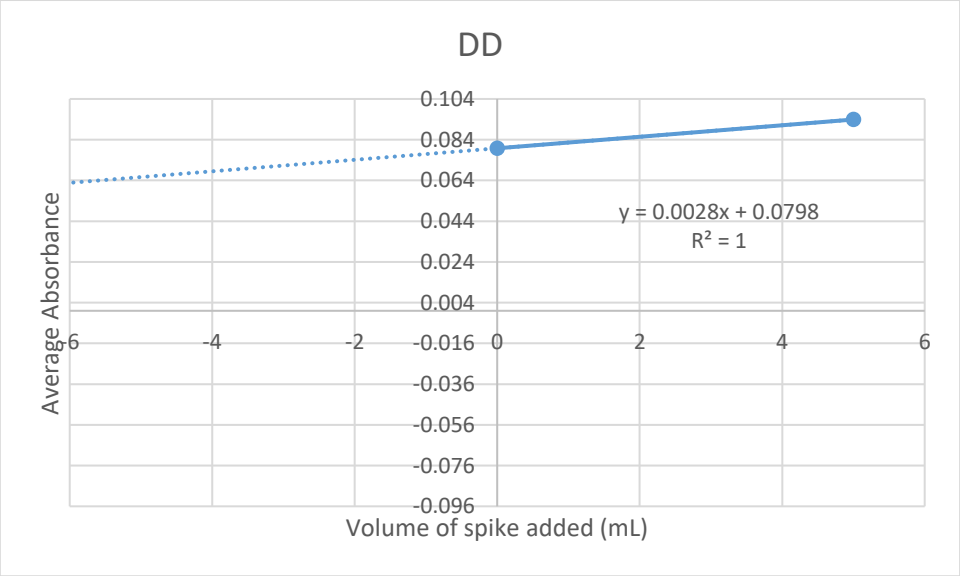

Figure 23: Standard addition curve for zinc of DD sample

Appendix C: Method Validation

Calculation of LOD and LOQ:

The SD of the 0 ppm iron standard was 0.000282 and the LOD for iron was determined as follows:

$$\frac{3 \times 0.000282}{0.0553} = 0.015\ ppm$$

To determine the LOQ for iron, the following equation was used.

$$\frac{10 \times 0.000282}{0.0553} = 0.05\ ppm$$

Calculation of precision criteria

Sample calculation for the precision criteria for iron (CR-2) sample was done as follows

CR-2 A	0.50
CR-2 B	0.58
CR-2 B	0.64
CR-2 A	0.61
CR-2A	0.62

Mean of CR-2 sample was calculated by: $\bar{x} = \frac{0.50+0.58+0.64+0.61+0.62}{5} = 0.59$

Standard deviation was calculated as follows: s= $\sqrt{\frac{(0.50-0.59)^2+(0.58-0.59)^2+(0.64-0.59)^2+(0.61-0.59)^2+(0.62-0.59)^2}{5-1}} = 0.056$

Relative standard deviation was calculated by: $\frac{s}{\bar{x}} \times 100\% = \frac{0.056}{0.59} \times 100\% = 9.53\%$

Variance was determined by: $s^2 = (0.056)^2 = 0.003$

Standard error of the mean was calculated by: $S_m = \frac{s}{\sqrt{N}} \Rightarrow \frac{0.056}{\sqrt{5}} = 0.025$

Accuracy calculation:

The concentration of added spike for Iron was 5 ppm.

The concentration of the spiked sample was determined the same way as done for the samples of the calibration method. Absorbance obtained from the AAS measurement for CR-AC-A sample : 0.2287

Corrected absorbance=> $0.2287 - 0.0138 = 0.2149$

Equation of the calibration curve => $y = 0.0553x + 0.0039$

Concentration of CR AC-A => $0.2149 = 0.0553x + 0.0039 \Rightarrow x = \frac{0.2149-0.0039}{0.0553} = 3.82$ ppm

The accuracy calculation for iron sample CR-AC-A was done as follows:

$$\%\ recovery = \frac{C_{spiked\ sample} - C_{unspiked\ sample}}{C_{added}} \times 100\%$$

The percentage recovery for sample CR-AC-A is done as follows: $\frac{3.82-0.67}{5} \times 100\% = 63\%$

The percentage recovery for the other samples and other metals is determined in the same way.