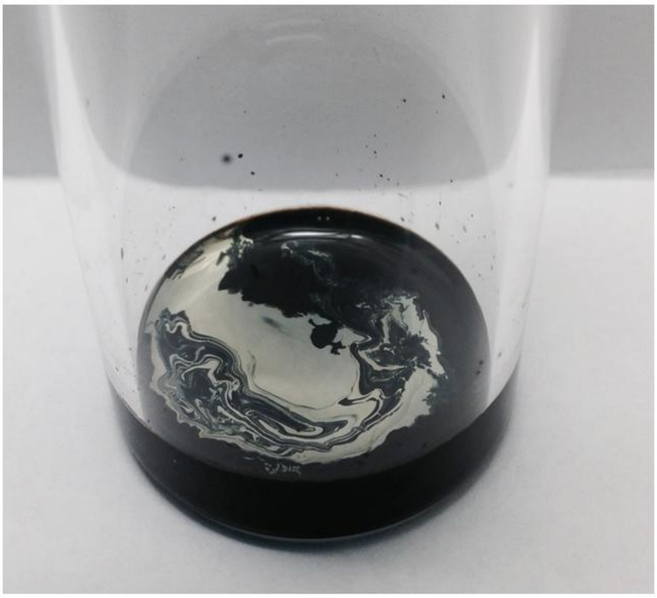

# Determination of Silver in Silicone Wound Dressings by Inductively Coupled Plasma - Optical Emission Spectroscopy

*Master of Science Thesis in Materials Chemistry and Nanotechnology*

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Gothenburg, Sweden, 2015

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## Abstract

In the late 20th century silver was first incorporated into commercial wound dressings as an antimicrobial substance. Mölnlycke Health Care produces and sells wound care products and disposable supplies for surgical operations. They have a wide range of silver containing products that demands accurate and fast analytical methods to aid the product development and comply with regulatory demands.

This work focused on improving and validate an inductively coupled plasma – optical emission spectroscopy method where the amount of silver in silicone wound dressings was determined with inductively coupled plasma. The sample preparation method used today is time consuming and developing a more efficient method would increase through-put, reduce costs and increase safety due to reduced handling of acids. During this work, the use of internal standard as volume marker and to control system variations was investigated together with an evaluation of the sample preparation methods. The final method was compared to the original and validated according to Mölnlycke Health Care's internal guidelines.

The final method was developed where dodecylbenzene sulfonic acid was used to digest the silicon dressing and hydrochloric acid to extract the silver. Indium showed good results as a volume marker and was also found to adequately compensate for system variations. The method has an increased safety and also an increased through-put which will speed up the product development. The final method showed insignificant difference compared to the original and was approved according to the validation guidelines at Mölnlycke Health Care.

**Keywords**: Dodecylbenzene sulfonic acid, ICP-OES, internal standard, silicone, silver.

# List of abbreviations

| | |
|---|---|
| CMC | Carboxymethyl cellulose |
| DBSA | Dodecylbenzene sulfonic acid |
| DOE | Design of experiments |
| HMDS | Hexamethyldisiloxane |
| ICP-OES | Inductively coupled plasma - optical emission spectroscopy |
| IS | Internal standard |
| LOD | Limit of detection |
| MHC | Mölnlycke Health Care |
| PTFE | Polytetrafluoroethylene |
| SDS | Sodium dodecyl sulfate |

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# 1. Introduction

Wounds are a preferable environment for bacteria and other microorganisms to grow in numbers. Due to this the healing of the wound may be delayed and there is a possibility for infections that may lead to life threating illnesses (Parsons, et al., 2005). To avoid bacterial growth, antimicrobial agents can be used in wound treatments. Currently, many different medical-device companies are incorporating silver into their products to make them antimicrobial.

Mölnlycke Health Care (MHC) produces and sells wound care products and disposable supplies for operations. A large portfolio of silver products demands accurate and high-quality analytical methods. The existing method is time consuming and sometimes needs to be outsourced to external companies at high cost. Developing a more efficient method, that could be undertaken in-house, would be both time saving and reduce the costs. One of the great challenges when measuring silver content is to extract the silver from the silicone dressings. To analyze the extracted silver the method used today is inductively coupled plasma optical emission spectroscopy (ICP-OES). The sample preparation method for the soft silicone containing product Mepitel Ag will be investigated during the work.

ICP-OES is used to measure trace elements in minute concentrations in liquids, down to µg/kg. ICP-OES uses argon gas to create a plasma that excite atoms and ions from the sample. These excited atoms and ions will radiate wavelengths during de-excitation that is specific for each element. The radiation will reach the detector, where the intensity of the signal can be related to the concentration of the chosen element. An advantage with ICP is its multielement capability, a property that few analytical techniques have (Thompson & Walsh, 1989) (Vogiatzis & Zachariadis, 2011).

During an ICP analysis the result can be affected by a variety of factors that are not possible to control. To eliminate some of these, an internal standard (IS) can be used. IS can act as a volume marker where it eliminates the result's dependency on changes in preparation volume. There are numerous alternatives when it comes to internal standards. Two of the most common are yttrium and indium and together with rhodium and lanthanum these were initially evaluated (Mokgalaka, et al., 2002) (Scheffler & Pozebon, 2013) (Grotti, et al., 2003).

## **1.1** Objectives of this study

The aim of the project is to improve and validate an ICP-OES method for determining silver content in silicone dressings. The first part of the work will involve the investigation of a suitable internal standard that can both act as a volume marker and compensate for changes in the ICP-OES system. To study how well the IS compensates, a design of experiment will be set up. In the second part of the project, a suitable sample preparation method will be developed by first downscaling the existing method and evaluate a better way to dissolve the silicone and extract the silver. The final method will be validated and tested on products. A successful outcome from the improvement will increase the through-put, reduce the costs and increase the safety due to handling of smaller volumes of acid. The work will also allow MHC to gain deeper understanding of their ICP-OES equipment.

The objectives can be summarized into four categories:

- Evaluate use of internal standard
- Evaluate sample preparation
- Validation of method
- Product testing

# 2. Theory

In this section the theory behind the thesis work is presented where silver, silicone, ICP-OES, internal standard and the sample preparation techniques used during the work is presented. At the end, design of experiments will be discussed.

## **2.1** Silver as antimicrobial substance and its chemistry

Silver as an antimicrobial substance have been known since ancient times when Hippocrates, father of western medicine, first described the use of silver and its antimicrobial properties in 400 B.C. (Owens, 2013). However, during that time it was not known how the silver acted on the bacteria. The bactericidal properties were first investigated in 1869 by Ravelin but it was not until the late 20th century that silver was first incorporated into commercial wound dressings (Thomas, 2010). Silver can exist as $Ag^0$ (metallic silver), $Ag^+$, $Ag^{2+}$ and $Ag^{3+}$, but the two higher oxidation states only form unstable complexes and is not applicable as antimicrobials (Lansdown, 2010). $Ag^0$ is not active in its metallic form but in contact with moisture $Ag^0$ is oxidized to $Ag^+$ and becomes antimicrobial (Lansdown, 2002). Silver has a low toxicity towards humans and is only needed in small quantities to be effective against a broad range of microorganisms (Percival, et al., 2005). In vitro studies have shown that 1-50 ppm of $Ag^+$ is needed for cytotoxicity (Malmfors, 2012). In the body, the silver ions will react with thiol groups of proteins in bacteria that are essential for the metabolic pathways and form complexes (Thomas, 2010). In the bacteria, the silver will make the cell walls more permeable (Owens, 2013).

Due to an increasing use of silver in commercial products the toxicity of silver has been investigated, but the results are not clear and can be interpreted in different ways (Malmfors, 2012). In humans the effects shown is accumulation at the skin, resulting in a discoloring called argyria. The effects are more a cosmetical concern than life threatening and there is no evidence of carcinogenicity or mutagenicity. Some cases of sensitivity and allergic reactions against silver have been found. At MHC recommendations have been made were the dose should not exceed 10 mg/kg/day provided that the exposure does not exceed 30 days (Malmfors, 2012).

To be able to analyze silver by ICP-OES it needs to be dissolved and is therefore often kept in $HNO_3$ since $AgNO_3$ that is formed has a high solubility. Another reason for using $HNO_3$ is silver's stability towards photo reduction and 1-10000 mg/kg silver solutions can be stable for years in $HNO_3$ (1-5 %). Silver dissolved in HCl or $H_2SO_4$ will act differently since it may form insoluble AgCl and $Ag_2SO_4$ and precipitate. However, a high concentration of $Cl^-$ will form soluble silver chloride ions e.g. $AgCl_2^-$. These types of solutions are also photosensitive and will form metallic silver if exposed to UV light. To avoid formation of precipitation and metallic silver from HCl solutions it is recommended that the HCl level should not be lower than 2-10 % and the $Ag^+$ concentration should not exceed 10 mg/l (Gaines, n.d.).

There are different analytical wavelengths that can be chosen for evaluation of silver with ICP-OES. Previously at MHC, 224.874 nm, 243.779 nm, 328.068 nm and 338.289 nm have been used and therefore these will be evaluated during this work. The difference between these four lines is the sensitivity, 328.068 nm have the highest sensitivity followed by 338.289 nm, 243.779 nm and 224.874 nm.

## 2.2 Silicone

Silicone is a diverse group of material that can take many forms and can be found in many different applications (Lewicki & Maxwell, 2013). The material can exist as an elastomer, emulsion or oil but they all consist of siloxy monomers that create polysiloxane chains (figure 1) (Brassard, 2010). The repeating unit's structure consists of a silicone atom (Si) bonding with one oxygen (O) and two pendant groups; most commonly two methyl groups ($CH_3$). Depending on the type of pendant group the silicone can be modified to receive specific characteristics (Strong, 2006). Silicone is a useful material due to its flexibility, hydrofobicity and outstanding thermal stability (Arab & Nagash, 2015).

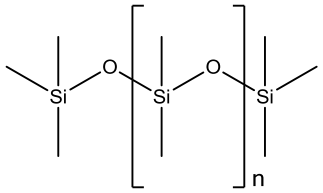

**Figure 1. One example of silicone. The picture shows polydimethylsiloxane.**

At MHC, silicone is used as an adhesive in wound dressings. For it to obtain desirable properties for this application it needs to be cross-linked. This can be done by addition curing. For the polymer chains to be able to cure the presence of functional groups, such as vinyl and hydride, is essential in the polymer chain. In the reaction a hydride-functional group is added onto a vinyl-functional chain in the presence of a catalyst, usually platinum (Lindgren, 2014) (figure 2).

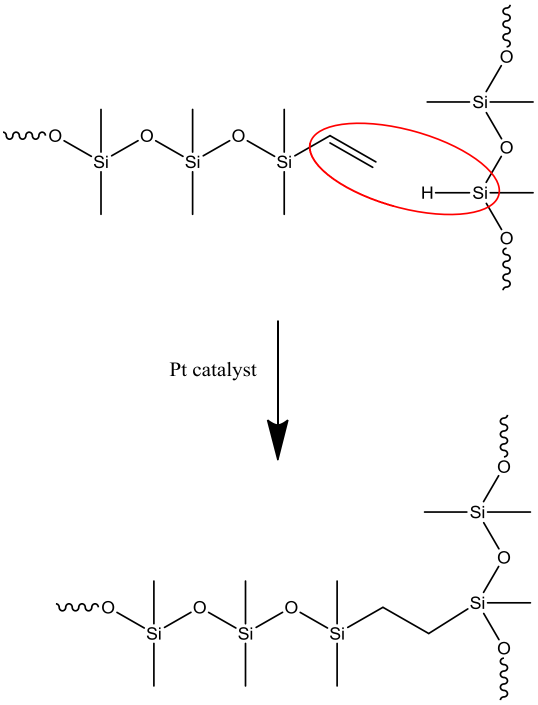

Figure 2. Addition curing with platinum as catalyst.

The silicone used is a so-called soft silicone that is prior to mixing divided into two components, A and B, where one of them contains the Pt - catalyst. The components are in some cases mixed with carboxymethyl cellulose (CMC). The silicone consists of smaller and larger polymers and the degree of cross-linking determines the properties of the material. The material can act both soft and hard depending on the shear force. For the Mepitel Ag products, the silver is incorporated into the silicone layer and is released upon contact with the moist skin and wound.

## 2.3 Inductively coupled plasma optical emission spectroscopy

The use of ICP-OES as an analytical technique was first described in 1964 by Greenfield and colleague (Greenfield, et al., 1964). After the mid 60's, the use and understanding of ICP advanced quickly and only ten years later several systems were commercially available. Nowadays, the greatest advances made in the field are improvements of computers used to analyze the ICP results. By using ICP-OES it is possible to measure silver contents down to μg/kg. A general picture of its components can be seen in figure 3.

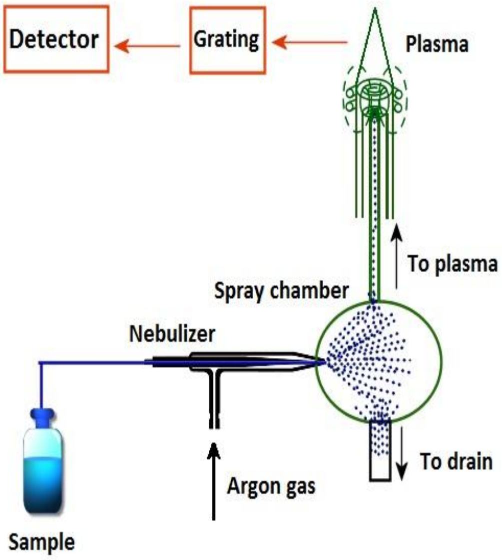

**Figure 3. The components of Inductively coupled plasma - optical emission spectroscopy.**

The ICP-OES system usually starts with an autosampler that introduces the sample to the nebulizer through a plastic tube. The sample is transferred by a peristaltic pump. The tubes used in this stage must be resistant towards the matrix used. The rate of the introduction can be varied by changing the peristaltic pump parameters such as the rate of the peristaltic pump, type of tubes or how hard the tubes are tightened onto the peristaltic pump.

For liquid samples the combination of a nebulizer and a spray chamber is the most common introduction system to the plasma. In the nebulizer the injector gas enters from the side, creating a region with low pressure, forcing the liquid sample to move towards the nozzle of the nebulizer and creating a fine aerosol together with the injector gas usually argon gas (figure 3). The choice of nebulizer depends on the characteristics of the sample but the most common, and also used during this work, is the concentric pneumatic nebulizer (Dean, 2005). Other choices are cross-flow or ultrasonic nebulizers. Cross-flow nebulizers have a wider capillary and can create an aerosol with samples that contains more particles without blocking the capillary. Ultrasonic nebulizers give an increased sensitivity but are more expensive and normally not used in routine analysis (Thompson & Walsh, 1989). Due to the small capillary in the concentric nebulizer there is a risk of blockage during analysis if there are any solid particles present in the sample.

The spray chamber will allow the appropriate amount of sample to reach the plasma. An introduction of a large amount of sample will cool the plasma or even extinguish it, leading to varying results. 2-4 % of the sample reaching the spray chamber will be analyzed in the plasma; the rest will be directed to the drain (Thompson & Walsh, 1989). During the analysis in this project the cyclonic spray chamber will be used where the aerosol is swirled and reduced to even smaller droplets before entering the plasma. Other types of spray chambers are double-pass or single-pass spray chambers. A double-pass will create a finer aerosol and increase the stability of the signal, but will take longer time to clean before the next sample is introduced (Dean, 2005). A single-pass chamber will give higher emission intensities but can decrease the signal stability (Todolí & Mermet, 2008).

Inside the plasma the temperature will reach 7000-10000 K and the atoms and ions from the sample will be excited due to collisions with electrons, argon ions and argon atoms. At the end of the plasma the temperature will decrease and a de-excitation of the excited electrons will occur. During de-excitation, energy will be released in the form of emitted light that is specific for each element. The wavelengths emitted from the atoms and ions can be viewed either axially or radially. Axial mode has better sensitivity than radial but the radial mode is not as dependent on the warm-up time of the plasma (Dean, 2005). The choice of viewing mode depends on the application. To light and sustain the plasma, an auxiliary gas consisting of argon is used.

The emitted light will reach the spectrometer which separates the emitted light into its wavelengths through a diffraction grating. The light will reach the detector which covers wavelengths between 167 to 852 nm. The diffraction grating consists of a series of lines that are tightly etched onto a mirror surface. When the light hits a line it is diffracted at a specific angle (Dean, 2005). With a computer software it is possible to choose specific wavelengths to analyze and evaluate. For ICP-OES, the signal is measured and presented in counts/second and a high amount of analyte gives a high number of counts. The recommended upper limit for ICP – OES varies but for the instrument used in this work it is at 300000 counts/second, after that the linearity will decrease and the result will not be reliable. The lower limit depends on the wavelength used during analysis and the amount of interferences.

The analysis can be affected by a variety of factors. A common cause leading to variations in the results is the composition and viscosity of the sample, so called matrix effects. Sample composition will affect the plasma temperature and hence the results. To eliminate this, the calibration standards are often made in the same type of matrix. However this is not always possible and might be time consuming. Another way to overcome the matrix effects is by dilution, but there is a risk of diluting below the limit of detection (LOD) (Scheffler & Pozebon, 2013). Nebulizer flow rate, auxiliary gas, tubing and pump rate can also affect the results since they affect the transportation of sample (Todolí, et al., 2002). Since some of these factors are not possible to fully control internal standard is commonly used (Sedcole, et al., 1986).

During analysis, so-called control samples are used to detect drifts in the system. The control samples is typically the middle level of the calibration curve which is injected at regular intervals during the analysis.

## 2.4 Internal standard

Internal standard is an element that is chemically and physically similar to the element that is analyzed. The IS should preferably be a rare metal that is not found in the sample. Yttrium, indium and rhodium are commonly used. A known amount of the internal standard is added to the sample either right before the introduction to the ICP-OES or before the sample preparation. If the IS is added during sample preparation it can be used as a volume marker, meaning that the sample analysis is no longer dependent on the sample preparation volume since the amount of analyte lost during preparation is equivalent to the amount of IS lost. The ratio between the IS and analyte will not change even though the volume of the matrix changes and thus the concentration, illustrated in figure 4. The internal standard may also be used to compensate for drifts in the instrument and also to eliminate the result's dependency of system changes, described in section 2.3 (Lorber, et al., 1984).

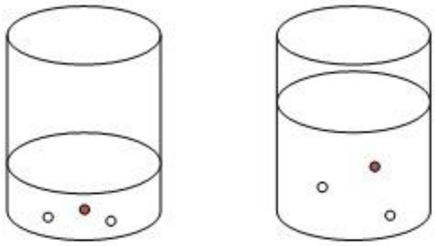

**Figure 4. An illustration of the use of IS as a volume marker. The ratio between internal standard (red) and analyte (white) is independent of the samples preparation volume.**

For evaluation the analyte/IS ratio is used to construct the calibration curve compared to the common case where the analyte concentration is used. It is important to add the same amount of IS to both the calibration standard and sample.

The analytical ICP-OES lines chosen for the internal standards during analysis of silver should be close to the silver line but not interfere. The ICP-OES consist of two diffraction grating plates, one with wavelengths between 167- 234 nm and the other with wavelengths between 235 - 852 nm. To obtain a better result, the chosen wavelength of the internal standard should be located on the same plate as the wavelength of silver.

## 2.5 Sample preparation

This step is important to achieve an accurate result. During sample preparation, the sample needs to be dissolved in a liquid without any interfering species or matrix residues. Ideally the preparation should be fast, minimize loss of analyte and minimize contamination (Brennan, 2008). A great challenge in this thesis is to digest the silicone dressing and extract the silver. During sample preparation two digestion techniques will be used, microwave digestion and open vessel digestion. Both techniques are different types of acid digestions where acids are used to digest the silicone and then extract the silver. In the original method, sulfuric acid is used for digestion and nitric acid for extraction of silver. As mentioned in section 2.1, HCl is another option for extracting silver from the matrix. HCl together with Ag is however more photosensitive and requires high $Cl^-$ concentrations, while $HNO_3$ is sensitive towards any presence of $Cl^-$ due to the formation of precipitations and loss of analyte.

### 2.5.1 Microwave digestion

Microwave digestion is carried out in a pressure-safe polytetrafluoroethylene (PTFE) vessel, called a bomb, with an accompanying ventilation cap. The bombs are arranged in a rotating circle and heated by microwave radiation. A metal vessel would absorb the microwaves and therefore the bomb must be made out of PTFE (Harris, 2010). However PTFE is sensitive towards concentrated sulfuric acid which is commonly used in sample preparation and in the existing method at MHC (Anton Paar, 2014). Inside the vessels higher temperatures can be reached due to an increase in pressure compared to other techniques, a feature that is the strongest reason for choosing this technique. The increase in temperature can be obtained faster with a microwave compared to an open vessel. Ramping of the temperature is crucial during method development since there is a possibility for the reaction to be exothermic and create a high pressure inside the vessel. If the pressure is too high the vessel cap will lift, release the excess pressure and then close again rapidly to prevent volatile products to escape (Dean, 2005). Microwave digestion is a rapid technique compared to open vessel digestion since the heat transfer is greater in a microwave compared to a heating plate or a heating block. Another advantage with microwave is that there is less risk of contamination since the vessels are closed (Brennan, 2008)

### 2.5.2 Open vessel digestion

In open vessel digestion the sample is typically placed inside a glass beaker and positioned in a heating block or placed on a heating plate. A heating block consists of several holes where the tubes can be placed. This will result in a more controllable temperature and a safer handling. Similar to microwave digestion the samples can be heated in stages by choosing a specific temperature program during method development. In open vessel digestion the samples can visually be monitored, a feature that is not possible with microwave digestion since the samples are inside an opaque vessel. However, it is not possible to reach as high temperatures as in microwave digestion.

## 2.6 Design of experiments

In order to investigate the influence of parameters on a response the classical approach is to use the one-factor-at-the-time approach (COST) until no further improvements can be detected. However, this approach requires many experiments and it is not possible to obtain information about the interactions between factors and identify the optimum settings (Rushing, et al., 2013) (Eriksson, et al., 2008). A more modern approach is design of experiments (DOE) where all relevant factors are varied simultaneously. DOE is useful for three different applications; screening, optimization and robustness. Screening will indentify which parameters that are most influential and how they should be evaluated. In the optimization application the most optimal operating conditions will be evaluated and during robustness tests the sensitivity for changes in parameters will be tested (Eriksson, et al., 2008).
The basis of DOE can be illustrated with a full factorial $2^n$ design where n defines the number of factors investigated. The reason it is called full factorial is because all possible extreme values of the design is tested. An evaluation starts by determining a standard reference experiment, so-called center-point, which are usually run in triplicates to detect variations. Full factorial experiments are then performed around these center-points where three factors (a, b and c) are varied in a symmetric cubic pattern (figure 5). If only two factors are varied, DOE is illustrated as a square with the extreme factor variations in the corners (figure 6) (Eriksson, et al., 2008).

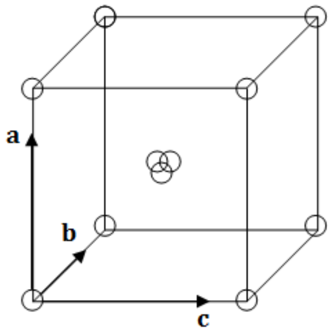

**Figure 5. $2^3$ full factorial design where 3 factors are varied.**

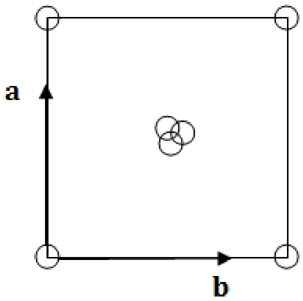

Figure 6. $2^2$ full factorial design where 2 factors are varied.

When the experimental data is obtained it will be evaluated using a computer software such as MODDE provided by Umetrics. The result will be presented in a contour plot were the relation between factors and response is illustrated (figure 7). During this work DOE will be used to investigate how well an internal standard compensate for system variations in the ICP-OES. The contour plot in figure 7 shows the dependency between the two chosen parameters, the nebulizer gas and the auxiliary gas. It also contains lines that illustrate the accuracies that are obtained with each setting. To detect a complete compensation, the contour plot should show a broad green picture with an accuracy at 100 %. To be able to say that there is no dependency between the nebulizer gas and auxiliary gas the lines should be vertical or horizontal. In figure 7 the lines are vertical which means that there is no dependency between the parameters.

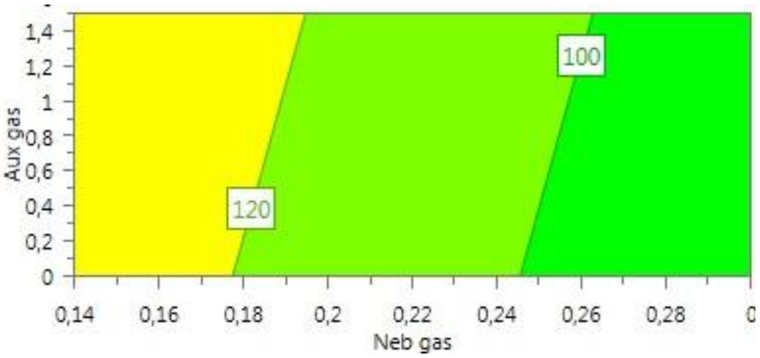

Figure 7. A contour plot showing the dependency between the changes in nebulizer gas and auxiliary gas and their effect on the accuracy.

# 3. Methods and results

In this section the experiments and results leading up to the final method is presented. First, an evaluation of internal standards was made where the interferences with the silver wavelengths were investigated. The use of internal standard as a volume marker and to eliminate system variation was also evaluated. The later was done with DOE. The internal standard evaluation was followed by the method development where swelling of silicone samples and digestion with Dodecylbenzene sulfonic acid (DBSA) were investigated. The use of microwave for digestion was investigated and it is presented at the end of methods and results since the evaluation was not completed. When the method was finalized, validation of method and product testing were performed.

## **3.1** Equipment

### 3.1.1 Instrument

For analysis, an ICP-OES iCAP 6200 DUO was used together with the computer programs iTEVA and Qtegra from Thermo Scientific. During digestion of silicone the samples were heated in Digitubes in a thermoblock SPB 50-48, both from Perkin Elmer. Samples were filtered through Digifilters with a vacuum pump connected to a manifold from Perkin Elmer or with glass microfibers filter from VWR. The evaluation of digesting silicone samples in a microwave were performed with a Multiwave GO from Anton Paar.

### 3.1.2 Chemicals

32 % Hydrochloric acid (Merck Millipore, Darmstadt, Germany)
65 % Nitric acid (Merck Millipore, Darmstadt, Germany)
96 % Sulfuric acid (Merck Millipore, Darmstadt, Germany)
70 % Dodecylbenzene sulfonic acid in isopropanol (Sigma Aldrich, United States of America)

30 % Hydrogen peroxide (Riedel – De Haën, Germany)

Sodium dodecyl sulfate (Sigma Aldrich, St. Louis, United States of America)

Carboxymethyl cellulose (Akzo Nobel, Arnhem, Netherlands)

Yttrium 1000 mg/l (Teknolab AB, Kolbotn, Norway)
Indium 1000 mg/l (Merck Millipore, Darmstadt, Germany)

Indium 10000 mg/l (Inorganic Ventures, Christiansburg, United States of America)
Lanthanum 10000 mg/l (Teknolab AB, Kolbotn, Norway)
Rhodium 1000 mg/l (Merck Millipore, Darmstadt, Germany)

Silver 1000 mg/l (Merck Millipore, Darmstadt, Germany)
Silver 10000 mg/l (Teknolab AB, Kolbotn, Norway)

Silver sulfate 98 % (Alfa Aesar, Ward Hill, United States of America)

Heptane (Merck Millipore, Darmstadt, Germany)
Isopropanol (Merck Millipore, Darmstadt, Germany)

### 3.1.3 Solutions

The solutions diluted during the work were prepared according to the following calculations:

$$\frac{Concentration\ of\ stock\ solution\ [mg/l]}{Desired\ concentration\ of\ solution\ [mg/l]} = Dilution\ factor$$

$$\frac{Final\ volume\ [ml]}{Dilution\ factor} = Volume\ taken\ from\ stock\ solution\ [ml]$$

The final volume was obtained by filling the beaker with water or acid.

## 3.2 Internal standard

It is important that the IS do not interfere with the analytical wavelength for silver. To investigate this, internal standards were combined with silver at different concentrations and analyzed at four common analytical wavelengths for silver (224.874 nm, 243.779 nm, 328.068 nm and 338.289 nm). The corresponding lines for the internal standards were chosen based on their intensities and position on the grating plate in the detector. The glassware used during the experiments was rinsed with acid prior to use to eliminate the risk of contamination of silver and other metals from previous experiments.

To evaluate interferences samples with silver at five levels (50 mg/l, 10 mg/l, 2 mg/l, 0.4 mg/l and 0.1 mg/l) with 2.5 ml of indium (1000 mg/l, 2.5 mg), 2.5 ml of rhodium (1000 mg/l, 2.5 mg) and 0.5 ml yttrium (1000 mg/l, 0.5 mg) was prepared in volumetric flasks.  The solutions were mixed from 1000 mg/l stock silver solutions and internal standards and diluted with ~0.5M $HNO_3$. The solutions were transferred to falcon tubes and analyzed with ICP-OES. By looking at the spectrums it was possible to detect interferences between the silver and IS (figure 8). To further investigate which IS was responsible for each interference; separate internal standard samples were prepared.

Separate silver solutions with the same amounts of yttrium, indium, rhodium as previous and also 250 µl of lanthanum (10000 mg/l, 2.5 mg) were tested to investigate interferences. To eliminate the possibility that there is any interference from the acid a separate test was performed where ~6 % $HNO_3$ was analyzed with ICP-OES. No interference was found. Figure 8 shows interferences were the yttrium and rhodium peaks overlap with the large silver peak.

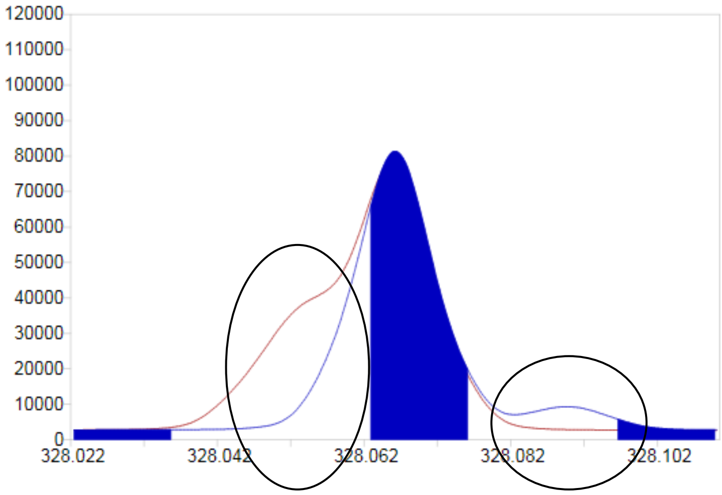

**Figure 8. Overlay of spectrums for yttrium and rhodium. The middle peak is silver at wavelength 328.068 nm, the red peak is yttrium and the blue peak is rhodium.**

The results from the two experiments are summarized in table 1. Based on the results, it was decided that indium and yttrium was best suited for further experiments. Due to limitations in the software, evaluation of multiple wavelength would have been time consuming and therefore only two, 243.779 nm and 338.289 nm, were chosen for further experiments.

**Table 1. Summary of the interferences at wavelengths 224.874, 243.779, 328.068 and 338.289 nm**

| Wavelength | Indium | Yttrium | Lanthanum | Rhodium |
|---|---|---|---|---|
| 224.874 nm | | | | Interference |
| 243.779 nm | | | Interference | Interference |
| 328.068 nm | | Interference | Interference | Interference |
| 338.289 nm | | | | |

### 3.2.1 Evaluation of internal standards by design of experiment

As mentioned previously, the use of IS can eliminate the effect of system variations in the ICP-OES system resulting in a more accurate analysis. To evaluate this, the flow of nebulizer gas, auxiliary gas and acid matrix were changed to simulate possible changes in the ICP-OES system. The effects were studied at two different silver concentrations, 0.4 and 10 mg/l. The nebulizer gas was varied between 0.14 and 0.3 ml/min since these are the lowest and highest values possible to use and still get good signal intensities. The auxiliary gas was varied between 0, 1 and 1.5 l/min. The matrixes chosen for this experiment was 10 % $HNO_3$, 15 % $HNO_3$ and aqua regia (30 % HCl and 10 % $HNO_3$), together with 200 µl yttrium (1000 mg/l, 0.2 mg) and 500 µl indium (10000 mg/l, 5 mg). The amount IS used during evaluation was chosen based on the relative intensity of the signal. A high intensity will result in a small amount of IS used and a low intensity demands a higher amount. Samples and combinations of settings were prepared according to the MODDE model (see appendix). The sample containing 5.2 mg/l silver solution was evaluated as a center-point and represents standard settings on the ICP-OES.

Six samples were prepared in volumetric flasks, where half of them contained the lower silver concentration and the rest the higher concentration. The flasks were then filled with either 10 % $HNO_3$, 15 % $HNO_3$ or aqua regia for the lower concentration and the same for the higher. The sample containing 5.2 mg/l silver contained 10 % $HNO_3$. Both yttrium and indium were added to all seven samples. Also, a blank for each matrix was prepared with the same amounts of yttrium and indium as the samples

For analysis at 243.779 nm and 338.289 nm two calibration curves were prepared, one for each wavelength. 338.289 nm had a curve that included lower amounts of silver due to a higher sensitivity. A low amount of silver will give a higher number of counts at 338.289 nm and therefore it is possible to use this wavelength at low amounts of silver. Wavelength 243.779 nm had a curve that included higher amounts of silver since it is not as sensitive as 338.289 nm.

The calibration curve used for analysis at wavelength 243.779 nm contained the silver concentrations 0.5, 1.9, 7.1, 26.6 and 100 mg/l in 6 % $HNO_3$ and for wavelength 338.289 nm the concentrations 0.002, 0.12, 0.71, 4.2 and 25 mg/l in 6 % $HNO_3$ were used. Both curves contained indium and yttrium in the same amount as the DOE samples and were run with default instrument settings.

Also, two blanks were prepared for the calibration curves, one containing 6 % $HNO_3$ and the other containing 6 % $HNO_3$ together with yttrium and indium.

For each wavelength three different runs were made, one for each setting of auxiliary gas. During each run the nebulizer gas was changed manually depending on which sample was analyzed at the moment. The results from each sample and setting were compared to the calibration curves since they represent the original instrument settings. During evaluation, the results were also calculated without compensation with internal standard. The results were presented as accuracies, which were calculated based on the known amount of silver added to the samples. A 100 % accuracy means that all the silver added to the samples are recovered. If the accuracy is above or below 100 % it means that something is wrong and a variety of factors could be the reason.

The results were inserted into the MODDE model and analyzed using a 4D model, shown in figure 9 for wavelength 243.779 nm and figure 10 for 338.289 nm. Figure 9 shows that the result for indium and yttrium is independent of the auxiliary gas since the lines are almost vertical. The nebulizer gas is the parameter that affects the accuracy the most. Without an internal standard there is a greater dependency between the nebulizer gas and auxiliary gas. The matrix does not affect the outcome since the graphs looks similar for yttrium, indium and without IS. In the MODDE software it was possible to change the silver concentrations to see how and if the result was dependent on concentrations. However, changing the silver concentration did not change the result (results not shown). As figure 9 shows, yttrium is the best alternative to eliminate changes in the system with accuracies close to 100 % for all combinations and increase the accuracy. The colored lines are broader, meaning that the system is more robust and it is possible to change the nebulizer gas and still obtain approximately 100 % in accuracy.

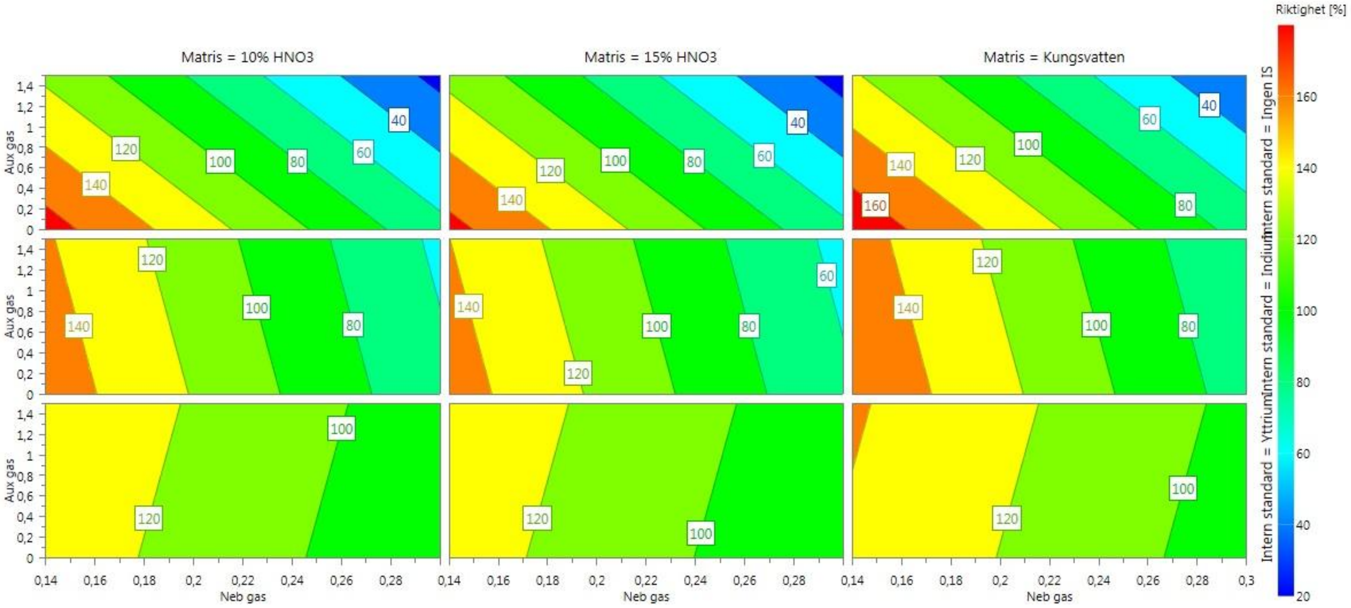

Figure 9. The results from DOE experiments with yttrium, indium and no IS. The result is presented in a 4D model for wavelength 243.779 nm.

The result for 338.289 nm is almost independent on both the nebulizer gas and the auxiliary gas for indium and yttrium. Without an internal standard there is a greater dependency between the nebulizer gas and auxiliary gas. Similar to 243.779 nm the result is independent of the silver concentration and matrixes. The same conclusion can be drawn about wavelength 338.289 nm as 243.779 nm; yttrium is the most suited IS since it gives the most robust result due to the broader lines and less change compared to the result without IS.

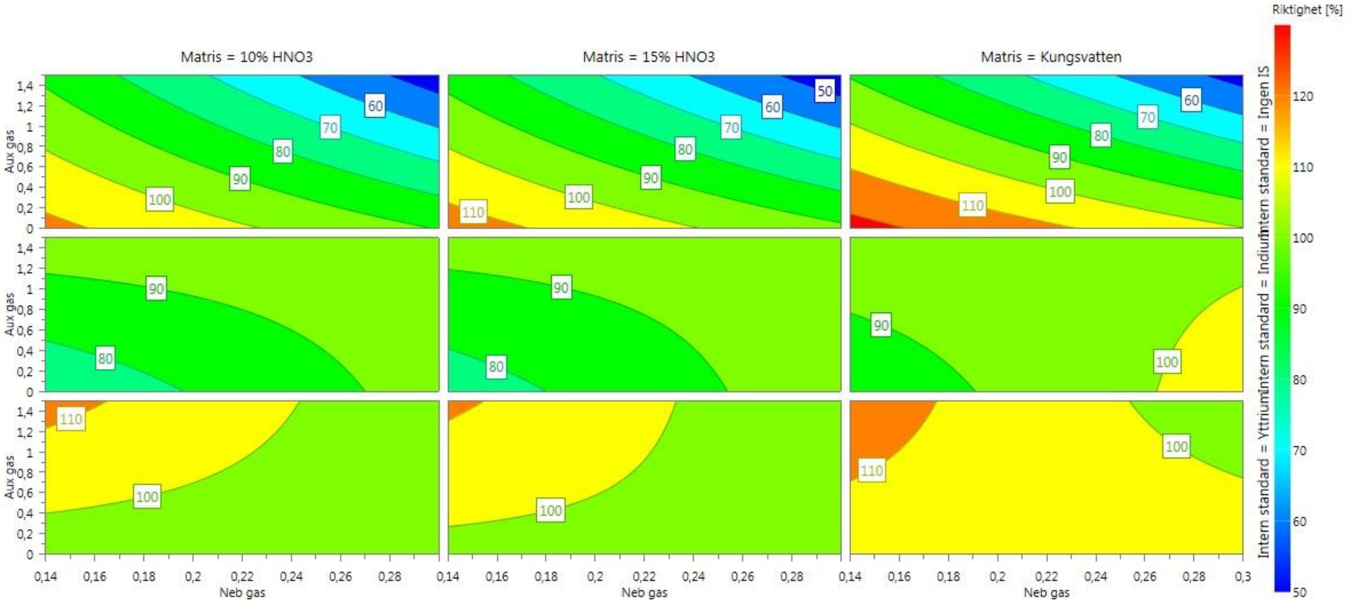

**Figure 10. The results from doe experiments with yttrium, indium and no IS. The result is presented in a 4D model for wavelength 338.289 nm.**

For both wavelengths the internal standards was not as optimal to compensate for the changes in system variations and matrixes as first expected, however it is clear that the use of an internal standard is beneficial. According to the results, yttrium will give the most robust system and will best eliminate the changes in the system variations for both wavelengths.

### 3.2.2 Evaluation of internal standard as a volume marker

An internal standard can also act as a volume marker as mentioned previously, which means that the analysis will be independent on the sample preparation volume or other volume changes.

To evaluate this, three Mepitel Ag samples were prepared by carefully removing ~0.05 g of the silicone surface of the product with a spatula and place it in glass tubes. The samples were swelled with hexamethyldisiloxane (HMDS) as described in section 3.3. The samples were digested according to the original method and were in addition down scaled five times as part of the method development work. 2 ml of 96 % sulfuric acid were added to the samples and heated at 175°C for 1 hour. 6 ml of 65 % $HNO_3$ and 100µl yttrium (1000 mg/l, 0.1 mg) were added to the digested samples and heated again for 30 min at 110°C. 10 ml of water was added to the samples and heated for an additional 10 min after the thermo block was turned off.

The samples were filtered through glass fiber filters and then diluted by mixing 1 ml of sample together with 6 ml of water, analyzed and evaluated with internal standard. The remaining volume of the samples were transferred to 100 ml volumetric flasks, diluted with water and then analyzed and evaluated without internal standard. The different steps will result in loss of analyte and sample preparation volume, therefore the exact volume is needed to be able to compare with the calibration curve and calculate the results without IS. The calibration curve used for this experiment was prepared the same way as in 3.2.1 and contained 0.005 mg, 0.02 mg, 0.1 mg, 0.5 mg and 2.5 mg Ag together with 100 µl yttrium (1000 mg/l, 0.1 mg) and 6 % $HNO_3$.

For these experiments the amount of silver was expressed as total content in mg instead of mg/l.

The result was evaluated with and without internal standard by comparing the obtained amount of silver with the mean value of silver content from the Mepitel Ag specifications (figure 11).

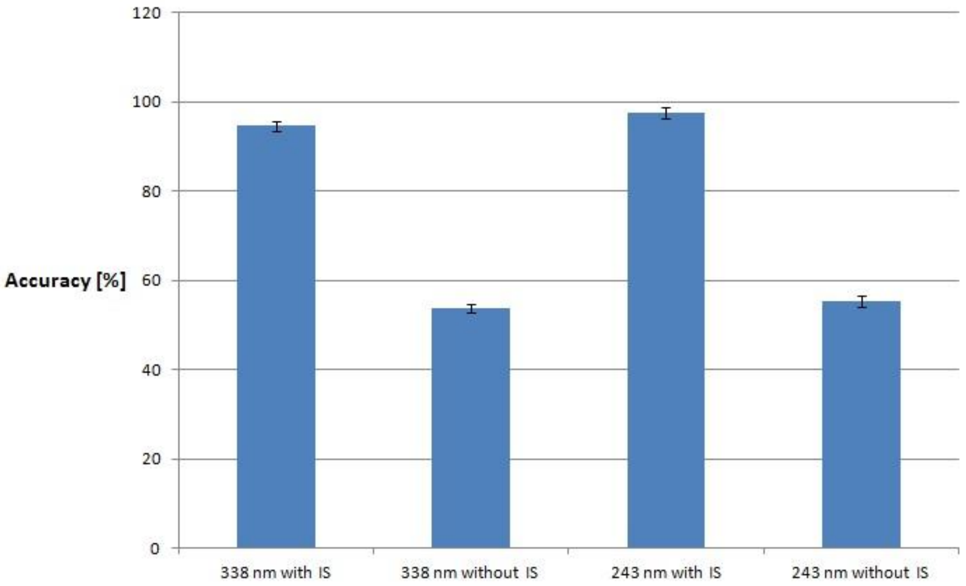

**Figure 11. A comparison between Mepitel Ag samples evaluated with and without internal standard (yttrium) for wavelengths 243.779 nm and 338.289 nm. n = 3, error bars show standard deviation.**

The results show that the internal standard improves the accuracy and that it is beneficial to use.

In the second experiment twelve silicone samples containing either stock silver solution or silver sulfate were prepared. By using stock silver solution it was possible to obtain low amounts of silver in the samples. The measurement uncertainties for weighing small amounts of silver sulfate are large and therefore weighing is not possible to use. However, for large amounts of silver it was not possible to use stock silver solutions since the liquid will prevent the silicone from curing properly. Therefore silver sulfate was used for higher amounts.

Six of the samples contained 10 µl of stock silver solution (1000 mg/l, 0.01 mg), ~ 0.005 g of CMC, ~ 0.05 g of silicone component A and ~ 0.05 g of silicone component B. The remaining six samples contained ~ 50 mg of 98 % silver sulfate, ~ 0.005 g of CMC, ~ 0.05 g of silicone component A and ~ 0.05 g of silicone component B. Two blanks containing ~ 0.005 g of CMC, ~ 0.05 g of silicone component A and ~ 0.05 g of silicone component B were also prepared. The samples were mixed thoroughly with plastic pipettes whose tips was cut off and added into the glass tubes. All fourteen samples were cured in 125°C for 40 min. Three samples from each set of six samples were swelled with 600 µl heptane and three were not swelled. All samples were digested as described earlier but contained 50 µl of yttrium (1000 mg/l, 0.05 mg). The samples were diluted by mixing 1 ml of sample with 6 ml of water in 15 ml falcon tubes. The remaining volume of the samples were transferred to 50 ml volumetric flasks and diluted with water up to 50 ml to allow evaluation without IS since the exact volume is needed to compare with the calibration curve and calculate the result. The calibration curve used for this experiment contained 0.025 mg, 0.095 mg, 0.355 mg, 1.33 mg and 5 mg Ag in 6 % $HNO_3$ for wavelength 243.779 nm and 0.001 mg, 0.006 mg, 0.0355 mg, 0.21 mg and 1.5 mg Ag in 6 % $HNO_3$ for wavelength 338.289 nm.

The samples from the 50 ml flasks were evaluated without IS and the samples in the tubes with IS. The samples containing silver sulfate (low sample) were analyzed at wavelength 243.779 nm and the others (high samples) at wavelength 338.289 nm due to different sensitivities. Results can be seen in figure 12 and only the results from the samples without swelling are presented.

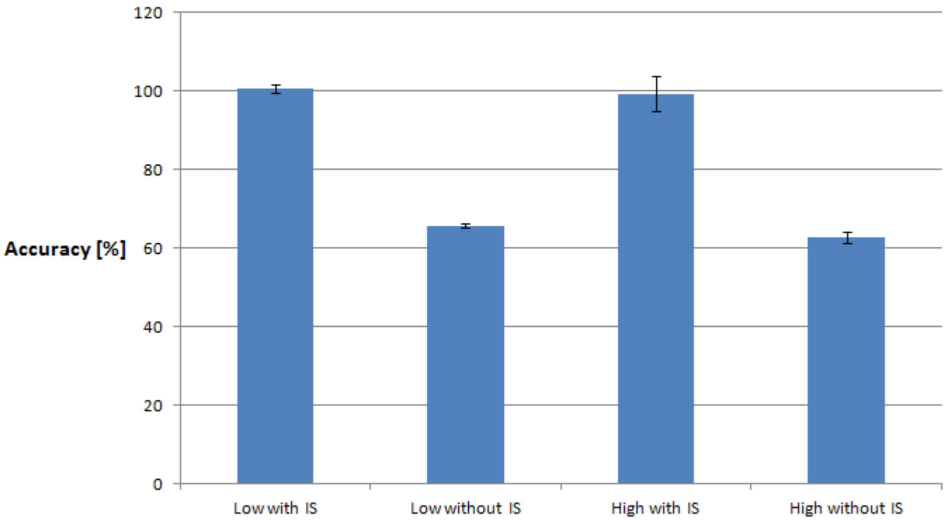

**Figure 12. A comparison between silicone samples evaluated with and without internal standard for low (0.01 mg) and high (50 mg) amounts of silver for wavelengths 243.779 and 338.289 nm. n = 3, error bars show standard deviation.**

The accuracy is improved by the use of internal standard.

## 3.3 Method development

### 3.3.1 Swelling of samples

To improve the contact between the cross-linked polymer chains in the silicone and the acid an idea was to swell the silicone. After consultation with Lars Lindgren at MHC, it was decided that hexamethyldisiloxane (HMDS), toluene, heptane and isopropanol could be suitable solvents for this purpose. One square centimeter pieces of Mepitel Ag and Mepitel was saturated in each solvent to study swelling. To make sure that it was not the CMC in Mepitel Ag that swelled, a small amount of CMC was also saturated in each solvent. As can be seen in figure 13, HMDS, toluene and heptane swelled the silicone, however isopropanol did not.

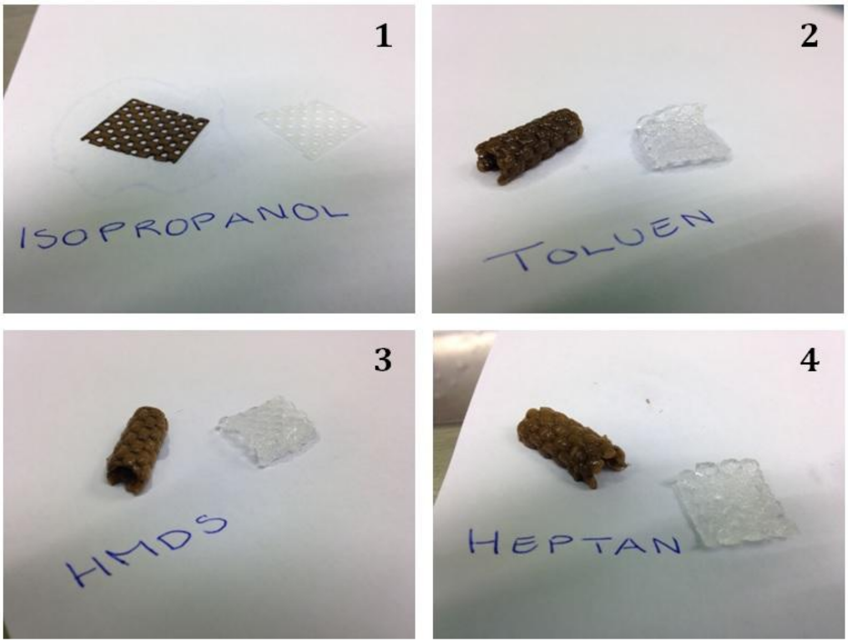

**Figure 13. Swelling of Mepitel Ag and Mepitel with isopropanol (1), toluene (2), hexamethyldisiloxane (3) and heptane (4).**

Toluene, HMDS and heptane were good swelling agents and were further investigated.

In the first experiment in section 3.2.2 three samples were swelled in HMDS and digested and analyzed. The results gave accuracies close to 100 %, which indicated that swelling is beneficial (figure 11).

In the second experiment in the same section, both swelled and non-swelled samples were evaluated and the results differed from each other. The results from the samples that were swelled had a smaller standard deviation and samples analyzed at wavelength 338.289 nm had a poorer accuracy if not swelled, a result that further supports the use of swelling (figure 14).

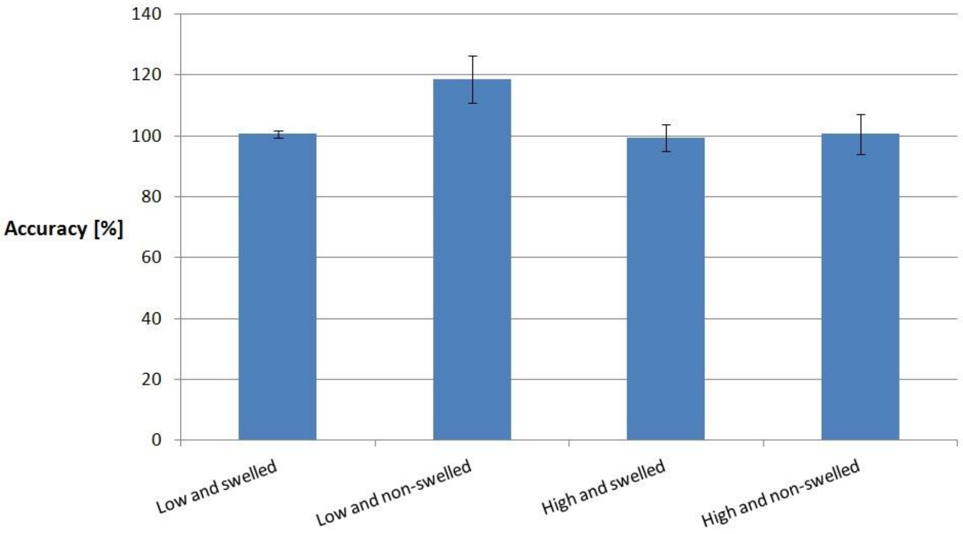

**Figure 14. Comparison between silicone samples containing low (0.01 mg) and high (50 mg) amounts of silver and either swelled or not swelled with heptane.**
**n = 3, error bars show standard deviation.**

Since swelling seemed to be a good option, other long chained solvents were tested. Dodecylbenzene sulfonic acid (DBSA) (figure 15) was investigated since it not only contains a long carbon chain but also is an acid that can possibly digest the silicone. It has previously been described to dissolve silicone (Haas & Snyder, 2004). Sodium dodecyl sulfate (SDS) (figure 16), is another option since it consists of a long carbon chain. Both SDS and DBSA were evaluated on six Mepitel Ag samples with digestion performed slightly different. For SDS, SDS was added as a salt to three Mepitel Ag samples together with 50 µl yttrium (1000 mg/l, 0.05 mg) and was then digested the same way as experiment two in section 3.2.2 but without heptane. For DBSA, 2 ml of DBSA was added together with 50 µl yttrium (1000 mg/l, 0.05 mg) to three Mepitel Ag samples. The three samples were then heated in a heating block at 175 °C and 6 ml of 32 % HCl was added to extract the silver. In this case HCl was used instead of $HNO_3$ due to a risk of nitration of the aromatic ring in DBSA that may create an explosive substance. After the first heating step the DBSA samples were fully digested and had a metallic phase on top of the sample (figure 17). The phase could possibly be silver in either metallic or ion form. Visually, it was possible to see that the sample was homogeneous and no remaining particles in the sample could be seen. After the addition of HCl a two-phase system was developed after a few minutes. The upper part consisted of a black oily organic phase and the lower of a water phase. After an additional heating step the black organic phase was removed and the samples were diluted and analyzed together with the SDS samples at wavelength 243.779 nm. The same calibration curves were used as in experiment two, section 3.2.2. The result is shown in figure 18.

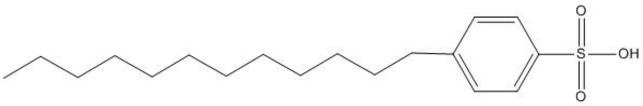

Figure 15. Structure of dodecylbenzene sulfonic acid.

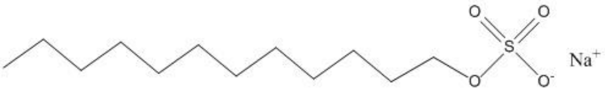

Figure 16. Structure of sodium dodecyl sulfate.

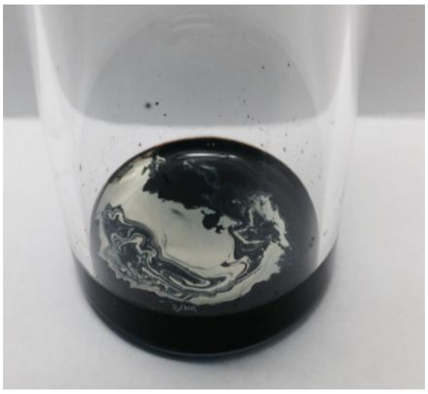

Figure 17. Metallic phase on top of the sample after digestion with dodecylbenzene sulfonic acid.

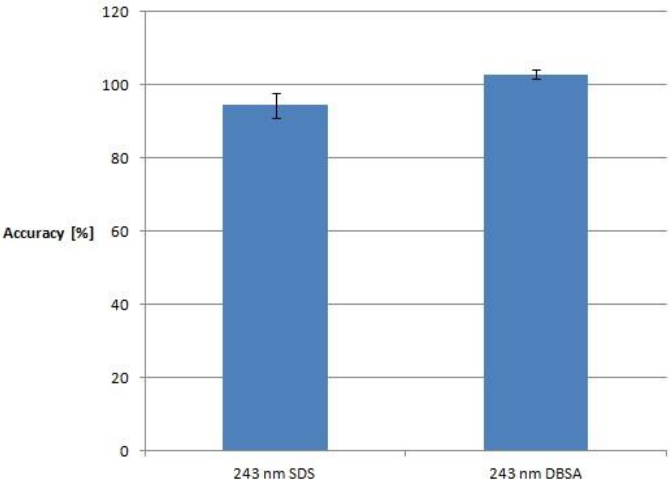

Figure 18. Accuracies for digestion of Mepitel Ag with SDS or DBSA analyzed at wavelength 243.779 nm. n = 3, error bars show standard deviation.

SDS did not show as good results as DBSA and the salt was hard to handle and was therefore not further evaluated. Due to the promising results obtained from the DBSA digestion further experiments were done to evaluate the accuracies in silicone samples since the previous experiment was performed with Mepitel Ag.

### 3.3.2 Digestion of sample with dodecylbenzene sulfonic acid

Seven silicone samples were prepared the same way as experiment two, section 3.2.2. Three of them containing low amounts of silver and three contained high amounts. One blank was prepared the same way. The samples were digested in DBSA similar to the Mepitel Ag samples in section 3.3.1 but yttrium was added after the first heating step. After the last heating step the samples were transferred to 50 ml falcon tubes and the glass tubes were rinsed twice with 6 ml of 32 % HCl. The falcon tubes were shaken before the upper organic phase was removed to make sure that all silver was extracted. 2 ml of the sample was diluted with 4 ml water in 15 ml falcon tubes. The calibration curves from experiment two, section 3.2.2 were used during analysis. The samples with low silver content were analyzed at wavelength 338.289 nm and those with high silver content were analyzed at 243.779 nm. The results can be seen in figure 19.

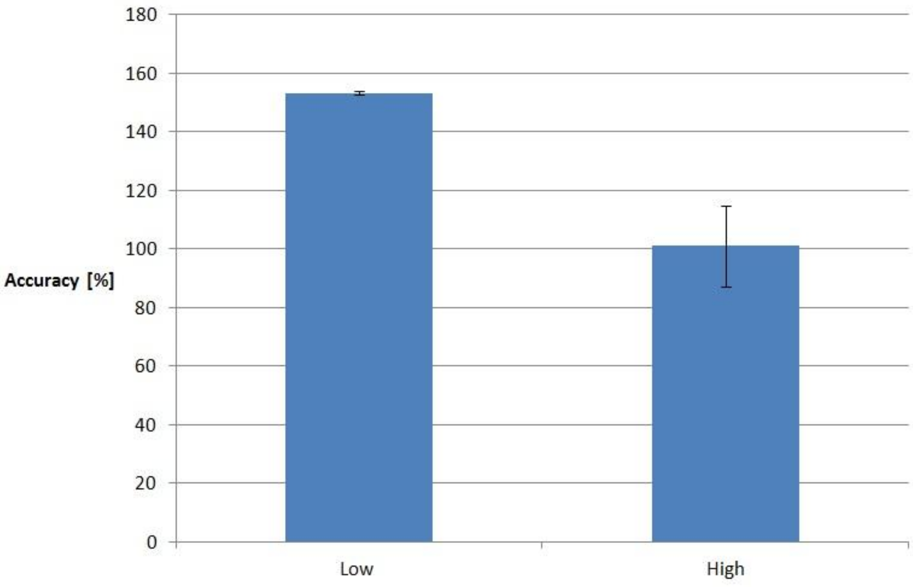

**Figure 19. Accuracy for samples with low (0.01 mg) and high (50 mg) amounts of silver digested in dodecylbenzene sulfonic acid at wavelengths 243.779 and 338.289 nm. n = 3, error bars show standard deviation.**

The results for DBSA samples at wavelength 243.779 were good. However, 338.289 nm showed poor accuracies. There is a possibility that an absolute amount of silver get stock on the tube wall during sample preparation and this is only noticed for low amounts of silver. To investigate this further, shaking of samples were evaluated.

#### *3.3.2.1 Evaluation of shaking*

In one experiment the time that the samples were shaken was studied. Twelve samples were prepared similar to section 3.2.2 where half of them contained stock silver solution and the other half contained silver sulfate. The samples were digested according to the previous experiment but yttrium was added before the first heating step and the second heating step had a temperature of 50°C. Three samples from each set of six were shaken for 90 seconds with a vortex and the other were not shaken (0 seconds). 1 ml of the samples was diluted with 9 ml of 10 % HCl. The calibration curves contained the following concentrations: 0.025 mg, 0.063 mg, 0.152 mg, 0.397 mg and 1 mg of Ag for wavelength 243.779 nm and the following for 338.289 nm: 0.001 mg, 0.006 mg, 0.0355 mg, 0.21 mg and 1.25 mg Ag in 10 % HCl. To achieve the most accurate results possible, the matrix in the calibration curve was changed to 10 % HCl since the samples were diluted with 10 % HCl. Doing this is believed to eliminate any matrix effects.

The samples with high silver content were analyzed at 243.779 nm and samples with low silver content at 338.289 nm. The results can be seen in figure 20.

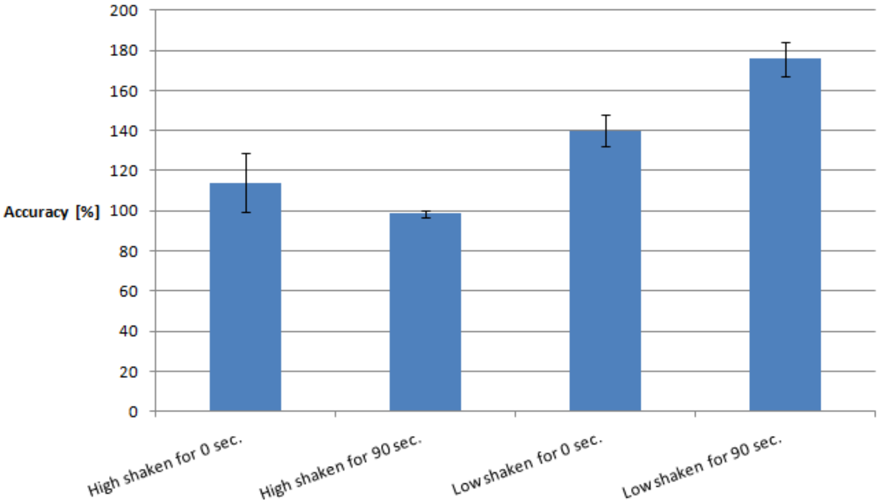

**Figure 20. Accuracies for comparison between samples with high (50 mg) and low (0.01 mg) amounts of silver, not shaken (0 seconds) or shaken for 90 seconds at wavelength 243.779 nm for high amounts and 338.289 nm for low amounts.**
**n = 3, error bars show standard deviation.**

The shaking had no effect on the results for samples with low silver content. However, for samples with high amounts of silver the results were improved by shaking 90 seconds also. The standard deviation also became better when shaking the samples in 90 seconds.

#### *3.3.2.2 Evaluation of addition of silver after heating*

To evaluate if the inaccurate results for wavelength 338.289 nm were due to uneven distribution of the IS, six samples were prepared according to section 3.2.2 but without any silver. The samples were digested with DBSA and transferred to 50 ml falcon tubes similar to the experiment described above but without any yttrium added. Before the samples were shaken from 90 seconds, 10 µl of silver (1000 mg/, 0.01 mg) together with 50 µl of yttrium (1000 mg/l, 0.05 mg) were added to three of the samples and silver together with 250 µl of indium (10000 mg/l, 2.5 mg) were added to the other three. Even though yttrium was a better choice than indium according to the DOE, indium was tested to evaluate if the high accuracies was dependent on the IS. The samples were analyzed at wavelength 338.289 nm and the same calibration curve was used as in the experiment above. The results can be seen in figure 21.

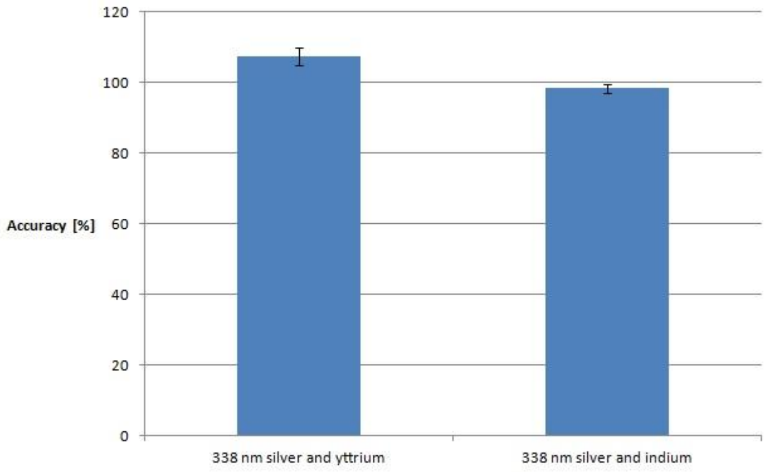

**Figure 21. Accuracies for addition of silver and IS after digestion of the silicone. n = 3, error bars show standard deviation.**

The results shows that addition of silver and IS after heating gave an accuracy close to 100 %. Therefore, the poor accuracies might be due to the heating. Indium however, showed promising results and the choice of IS seems to play a role. Due to these results, indium was included in the further experiments.

#### *3.3.2.3 Evaluation of evaporation of silver stock solution*

The poor accuracy that can be seen at wavelength 338.289 nm might be due to the low amount of silver and that it is close to LOD. Since it is desirable to use wavelength 338.289 nm due to a higher sensitivity compared to 243.779 nm, the lowest value on the calibration curve needs to be increased. To be able to have higher amounts of silver in the silicone samples more stock silver solutions needed to be added. However this was not possible since too much liquid would inhibit the cross-linking of polymer chains. An idea was to mix CMC with stock silver solution and evaporate the liquid.

To evaluate the idea, eight samples were prepared where three of them contained 250 µl of stock silver solution (1000 mg/l, 0.25 mg) and the other three contained 500 µl of stock silver solution (1000 mg/l, 0.5 mg). Two blanks were also prepared. The stock silver solution and ~ 0.005 g of CMC was mixed and were heated in a thermoblock at 90 °C until the liquid had evaporated. All samples were prepared and digested similar to experiment two section 3.3.2 together with 50 µl of yttrium (1000 mg/l, 0.05 mg) and shaken for 90 seconds. The samples containing 250 µl silver was analyzed at wavelength 243.779 nm and the samples containing 500 µl was analyzed at 338.289 nm. The same calibration curves as the experiment above were used. The result can be seen in figure 22.

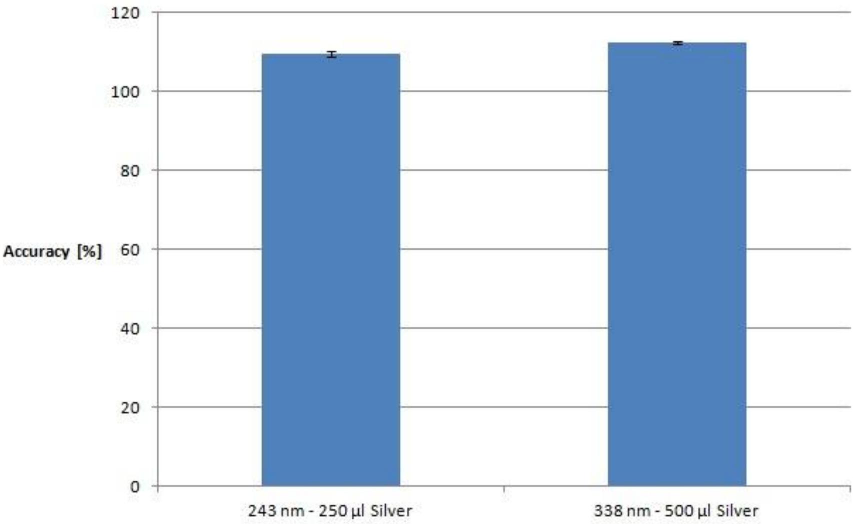

**Figure 22. Accuracies for silicone samples where stock silver solution at different volumes was evaporated before curing. n = 3, error bars show standard deviation.**

Evaporation of stock silver solution showed promising results and was used further on to be able to reach higher amounts of silver without compromising the curing of silicone.

### *3.3.2.4 Evaluation of indium as internal standard*

Indium showed promising results in experiment 3.3.2.2 and was therefore further evaluated. The samples containing low silver content have previously showed varying results and were therefore increased during this experiment.

Twelve samples were prepared and digested similar to the experiment above. The silicone samples were made with stock silver solution at two different concentrations for each wavelength. 30 µl of stock silver solution (100 mg/l, 0.003 mg) and 500 µl of stock silver solution (1000 mg/l, 0.5 mg) for wavelength 338.289 nm were used. For 243.779 nm, 75 µl and 500 µl of 1000 mg/l were used. The same calibration curves as above were used. Indium and yttrium were added to all samples. Results are shown in figure 23 for wavelength 243.779 nm and figure 24 for wavelength 338.289 nm.

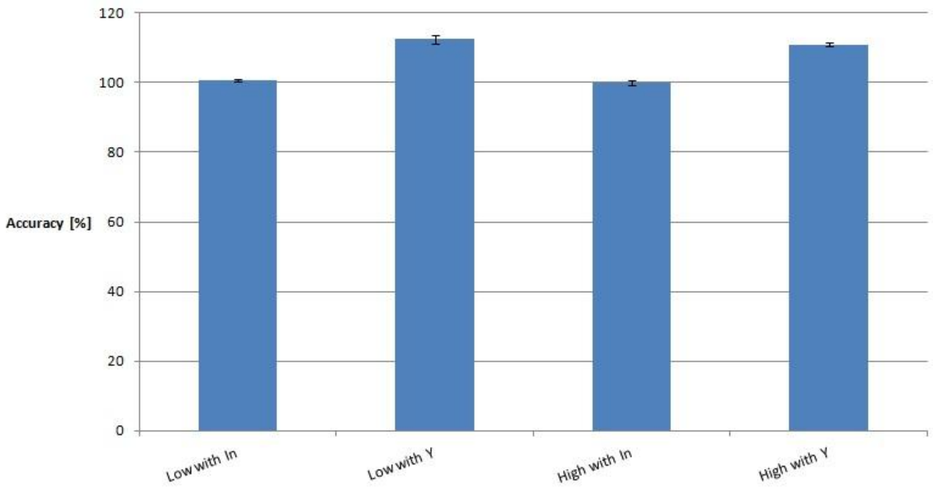

**Figure 23. Accuracies for comparison between indium (In) and yttrium (Y) as internal standard for low and high silver content analyzed at wavelength 243.779 nm.
n = 3, error bars show standard deviation.**

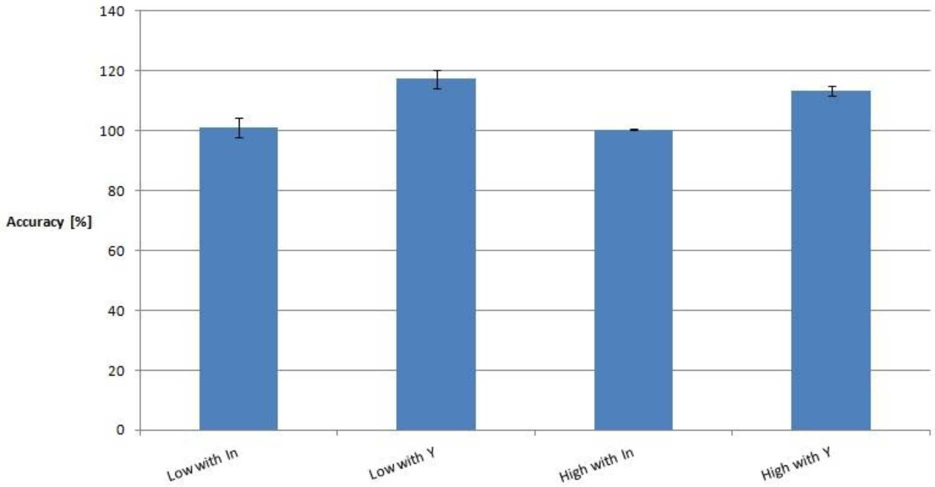

**Figure 24. Accuracies for comparison between indium (In) and yttrium (Y) as internal standard for low and high silver content analyzed at wavelength 338.289 nm.
n = 3, error bars show standard deviation.**

The experiment showed promising results for the current method and it was decided to use indium as an internal standard due to the results shown in figures 23 and 24.

#### *3.3.2.5 Evaluation of plastic Digitubes*

During the experiments all glassware has been rinsed with acid as mentioned before. This is a time consuming step and to eliminate this plastic tubes are an alternative. Digitubes would provide the possibility to inject the samples directly to the ICP-OES since the tubes contain volume marks that makes it possible to dilute directly in them. The plastic tubes need to resist temperatures up to 175°C since that is the temperature used in the first heating step.

A test was made to evaluate if it was possible to digest the silicone sample at 150°C instead since the temperature limit for Digitubes is 150°C. In the experiment, three Mepitel Ag samples were digested in 2 ml DBSA at 100°C, 120°C or 150°C. The result was visually evaluated and all three samples were dissolved.

An experiment was then done where glass tubes and Digitubes were compared. At the same time, different ways to dilute the samples were tested. Six silicone samples were prepared in glass tubes according to experiment four, section 3.3.2. Three of them contained 90 µl of stock silver solution (100 mg/l, 0.009 mg) and the other three 800 µl of stock silver solution (1000 mg/l, 0.8 mg). Twelve samples were prepared the same way in Digitubes, six of them contained the lower concentration and the other six contained the higher concentration. All samples were digested according to experiment two, section 3.3.2 but the first heating step the temperature was 150°C and the samples were shaken for 90 seconds. To the Digitube samples, 12 ml of 32 % HCl was added directly before being shaken. 1 ml of the samples in the glass tubes was diluted with 9 ml of 10 % HCl. Three of the Digitubes with lower concentration and three of the Digitubes with higher concentration were also diluted similar to the glass tubes for comparison. The remaining six Digitubes samples were diluted by removing most of the sample and leaving 5 ml in the tubes. The 5 ml were diluted with 10 % HCl up to the 50 ml mark and the samples were then injected directly in the ICP-OES. The samples were analyzed at wavelength 338.289 nm and the calibration curve used contained 0.003 mg, 0.013 mg, 0.055 mg, 0.235 mg and 1 mg of Ag in 10 % HCl. The analysis was performed only for wavelength 338.289 nm since the calibration curve was extended to contain the highest level for 243.779 nm as well. The result for indium can be seen in figure 25.

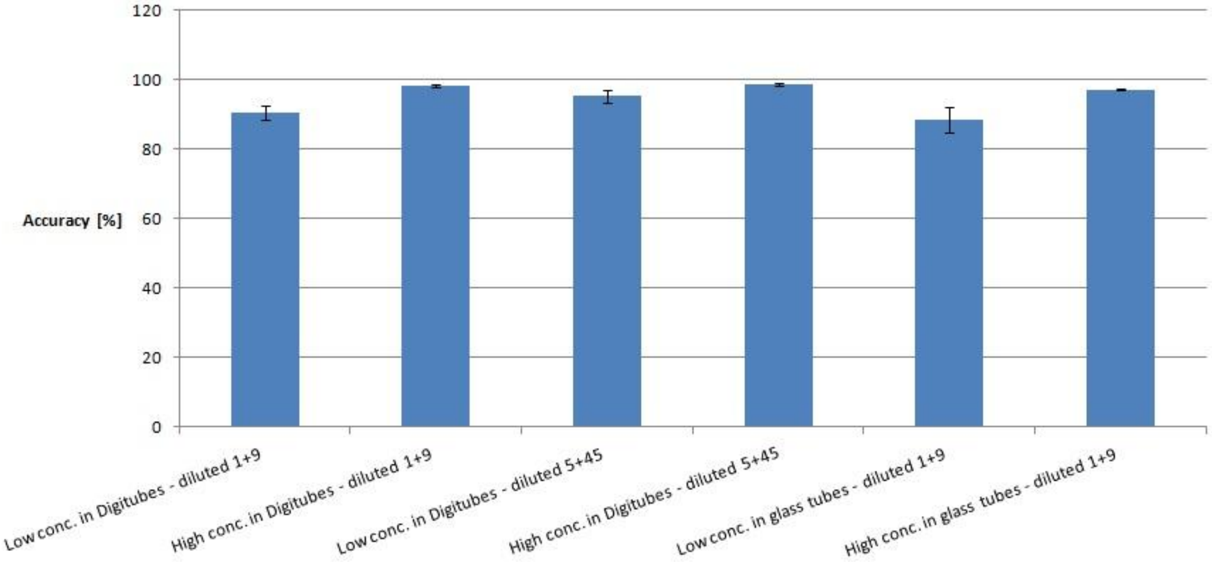

**Figure 25. Accuracies for comparison between glass tubes and Digitubes and comparison between different types of dilution. Indium is used as IS. The samples contain low and high amounts of silver. n = 3, error bars show standard deviation.**

The results from the comparison between Digitubes and glass tubes showed no major difference. However, the results for the low amount of silver had a low accuracy.

The samples above were re-run several times due to drifts during the ICP-OES analysis. It was noticed that the results varied depending on the warm-up time of the plasma. Longer warm-up time gave a result close to 100 %. In the result (figure 25) the plasma was warm and the control samples were stable during the analysis.

#### *3.3.2.6 Evaluation of reduced heating, time of shaking and filtering*

To further reduce the sample preparation time, the second 10 min heating step and the time of shaking was evaluated. Six silicone samples were prepared and digested according to the experiment above and shaken for 90 seconds. Three of the samples contained 270 µl of stock silver solution (100 mg/l, 0.027 mg) and the other three contained 800 µl of stock silver solution (1000 mg/l, 0.8 mg). Six more samples were prepared and digested the same way but were shaken for 30 seconds and without heating a second time. The samples contained the same amount of silver as previously. All twelve samples were filtered through Digifilters before analysis to obtain cleaner samples and possibly improve the accuracy. The calibration curve used contained 0.003 mg, 0.013 mg, 0.055 mg, 0.235 mg and 1 mg of Ag in 10 % HCl. The result is shown in figure 26.

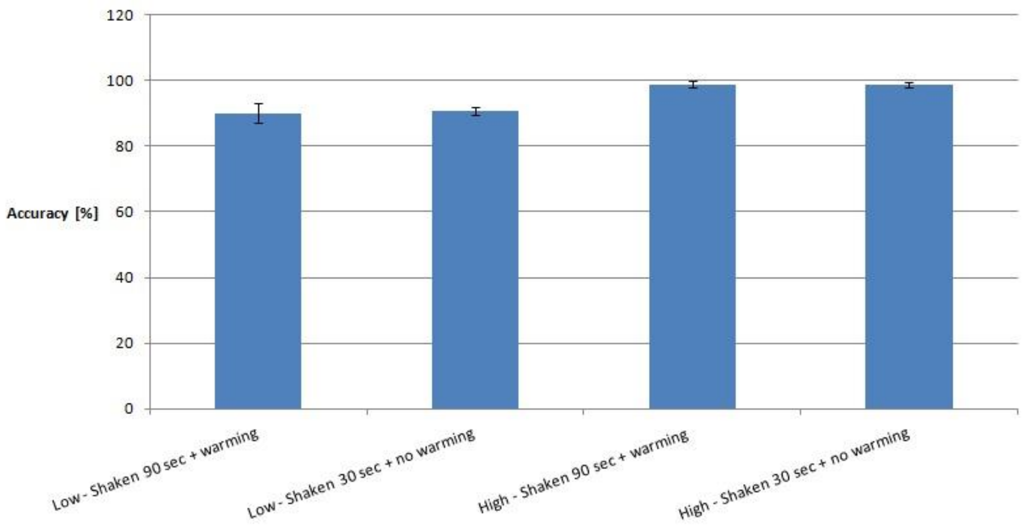

**Figure 26. Accuracies for comparison between samples shaken 0 or 90 seconds and comparison between heating for 10 min and not heating. All samples were filtered through Digifilters. Analyzed at wavelength 338.289 nm for low and high amounts of silver. Indium is used as IS.**
**n = 3, error bars show standard deviation.**

The results were similar with and without warming and shaking for different amounts of time. The accuracies were acceptable although a bit low for the low silver content, but showed that filtering is possible.

### 3.3.3 Evaluation of microwave digestion

At the beginning of the method development work, the use of microwave digestion was evaluated.

To evaluate the most suited work up solution for silicone digestion and the use of IS, six silicone samples were prepared in microwave digestion bombs. ~0.01 g of silver sulfate, ~0.01 g of CMC, ~0.1 g of silicone component A and ~0.1 g of silicone component B were mixed with the use of a plastic pipette whose tip was cut off and added to the sample. The samples were cured at 125°C in 40 min. To three of the cured samples 10 ml of 65 % $HNO_3$ was added together with 2.5 ml of indium (1000 mg/l, 2.5 mg). To the remaining three samples 10 ml of 65 % $HNO_3$, 1 ml of 30 % $H_2O_2$ and 2.5 ml of indium (1000 mg/l, 2.5 mg) was added. The samples were heated to 100° C, then the temperature was held for 10 minutes and then the samples were heated again for 10 minutes at 180° C. The number of heating intervals can be chosen but after digestion the samples were cooled down prior to opening. 1 ml of sample was diluted with 9 ml of ~6 % $HNO_3$. To evaluate the result without any internal standard the remaining volume of the sample was then transferred to a 50 ml volumetric flask and diluted with water up to the 50 ml mark. 2 ml of sample were transferred to 15 ml falcon tubes and diluted with 8 ml of ~6 % $HNO_3$ and analyzed. The calibration curve contained the following amounts of silver: 0.005 mg, 0.02 mg, 0.1 mg, 0.5 mg and 2.5 mg Ag in 0.5 M $HNO_3$. The result can be seen in figure 27.

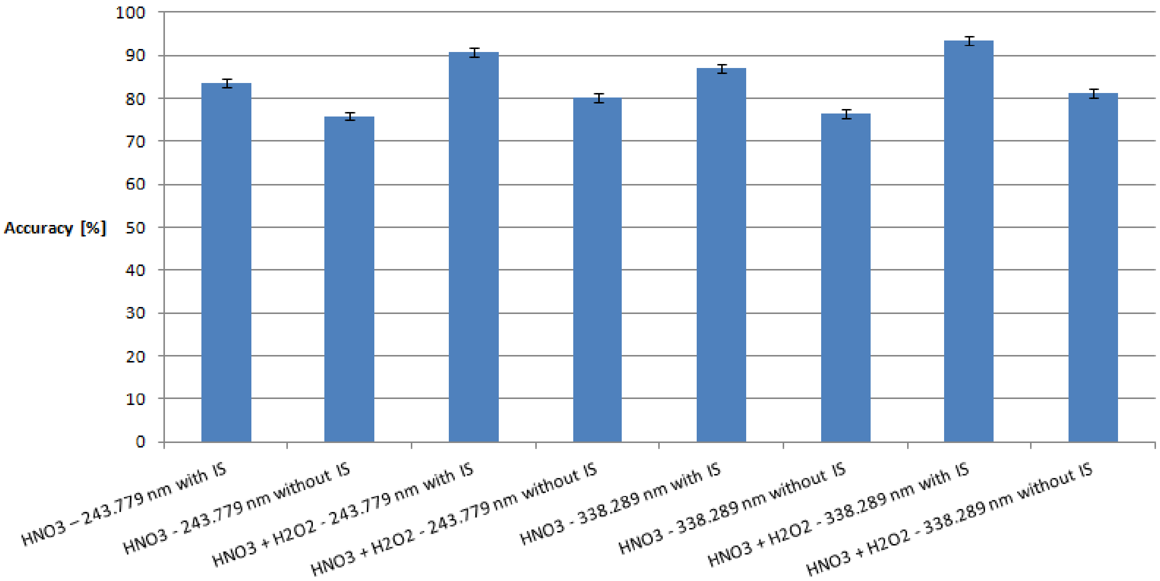

**Figure 27. Accuracies for evaluation of $HNO_3$ or $HNO_3$ together with $H_2O_2$ as work-up solution. Indium was used as internal standard. n = 3, error bars show standard deviation.**

$H_2O_2$ and $HNO_3$ together with an internal standard gave the most promising result and were used further on.

Inside the bombs it was possible to detect some silicone and silver remains that correlated with the accuracies and gave high standard deviations.

Swelling of samples in microwave bombs were also evaluated by using nine Mepitel Ag samples. Three of them were swelled in HMDS, three were swelled in heptane and the remaining three were not swelled for comparison.
The samples were digested with 65 % $HNO_3$ and 30 % $H_2O_2$ together with indium and yttrium. 1 ml of the digested samples were diluted with 4 ml of water and were filtered if needed. The calibration curve used contained 0.005 mg, 0.02 mg, 0.1 mg, 0.5 mg and 2.5 mg Ag in 6 % $HNO_3$. The samples containing HMDS had evaporated and were not possible to evaluate. The samples were analyzed at wavelengths 243.779 nm and 338.289 nm and the result can be seen in figure 28.

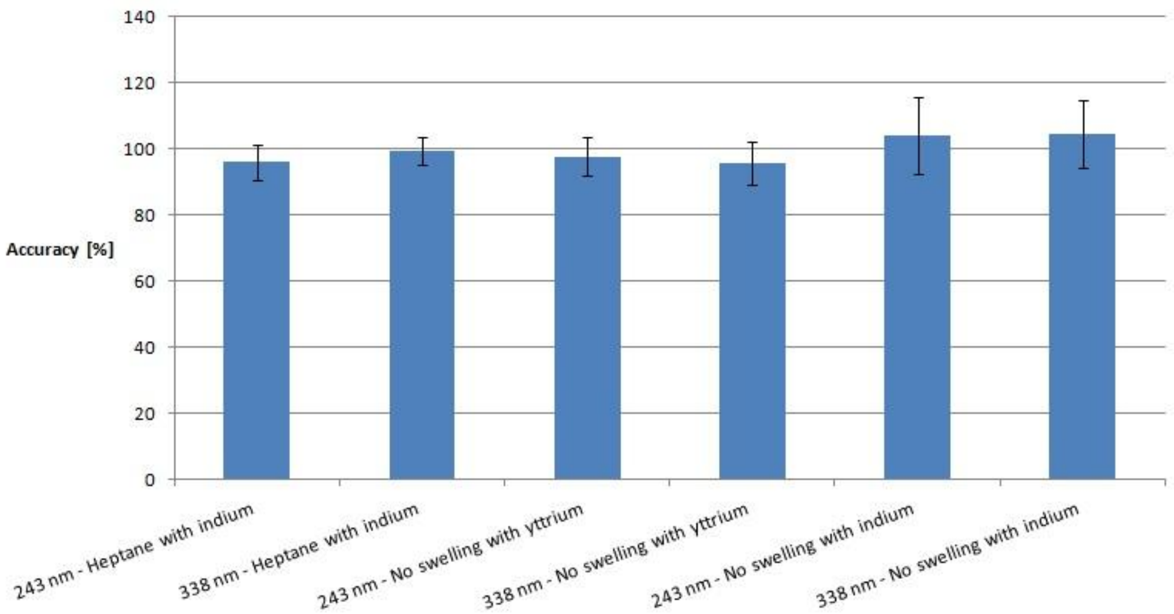

**Figure 28. Accuracies for comparison between samples swelled in heptane or non-swelled for wavelengths 243.779 and 338.289 nm. n = 3, error bars show standard deviation.**

The accuracies were promising but with a high standard deviation for some samples. That may be due to an insufficient digestion of the silicone, a feature that was observed in the previous experiment. No further experiments were done due to promising DBSA results.

## 3.4 Method validation

The method validation was performed according to MHC internal validation guidelines. A risk assessment was performed to identify possible hazards in the method and also a test plan was set up. In the validation guidelines, it was decided that three replicates should be prepared at three different levels, low, middle and high. Three people should do the same method to evaluate the robustness.

To validate the method, a calibration curve in silicone was prepared and the silicone was digested according to the current method. Even though the samples containing low silver content had been increased prior to method validation, the accuracies were poor (figure 26). By matching the matrices for the calibration curve and samples there is a possibility to eliminate the poor accuracies for samples with low silver content. The calibration curve samples contained ~0.005 g of CMC, ~0.05 g of silicone component A and ~0.05 g of silicone component B together with 400 µl of 67.5 mg/l, 235 mg/l, 825 mg/l, 2875 mg/l or 10000 mg/l stock silver solution. This results in a calibration curve with 0.027 mg, 0.094 mg, 0.33 mg, 1.15 mg and 4 mg Ag. CMC together with the stock silver solution were added to a Digitube and were evaporated in a thermoblock at 90 °C before the silicone components were added. The samples were mixed with a plastic pipette whose tip was cut off and added to the tubes. The samples were cured at 125°C for 40 minutes.

Nine control samples were prepared and cured similar to the calibration curve; three of them contained 0.027 mg of Ag, three contained 0.33 mg of Ag and the remaining three contained 4 mg of Ag.

To all silicone samples 50 µl of yttrium (1000 mg/l, 0.05 mg) and 250 µl of indium (10000 mg/l, 0.25 mg) were added together with 2 ml of DBSA. The samples were heated for 1 hour at 150 °C. 18 ml of 32 % HCl was added to each sample and the samples were shaken for 30 seconds. The organic phase together with most of the sample was removed and 5 ml was left in each tube. The samples were diluted with 10 % HCl up to 50 ml and filtered through Digifilters. The samples were analyzed at wavelength 338.289 nm and the results can be seen in figure 29.

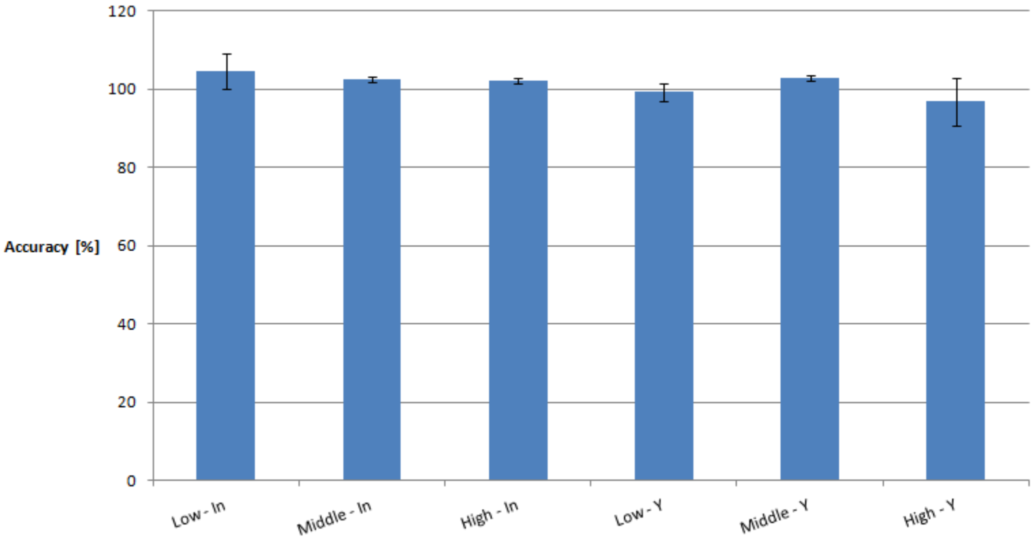

**Figure 29. Accuracies for validation of samples containing low (0.027 mg), middle (0.33 mg) and high (4 mg) amounts of silver for indium and yttrium. n = 3, error bars show standard deviation.**

To evaluate the possibility of storing the samples for a week, the validation samples were stored in the dark and re-run after 7 days and the results were similar, hence the samples can be stored (results not shown).

To evaluate the possibility to analyze products containing silver above the limit of quantification. Three samples were made that contained 6 mg silver. These were prepared and digested similar to the validation samples but was diluted differently. Instead of 5 ml, 2.5 ml of sample was left in the tubes and was diluted up to 50 ml with 10 % HCl. The samples were analyzed at wavelength 338.289 nm. The result can be seen in figure 30.

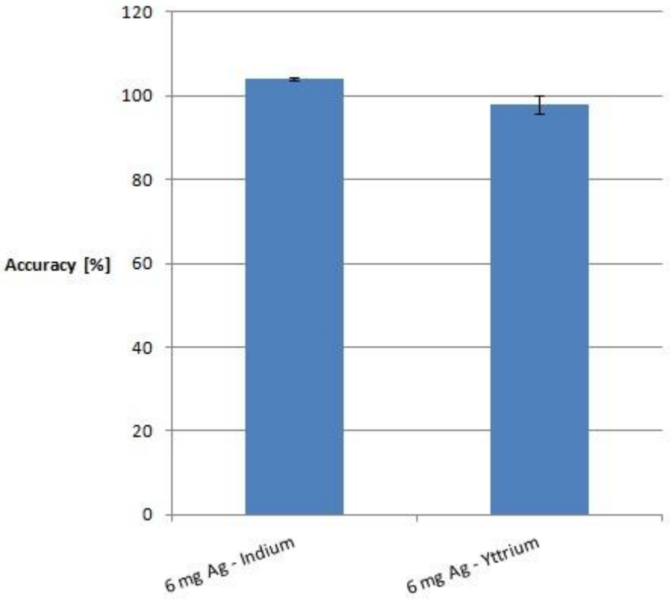

**Figure 30. Accuracies for samples containing high amounts of silver. n = 3, error bars show standard deviation.**

## 3.5 Product testing

To compare the current method with the original, ten samples of Mepitel Ag were digested with each method. The number of samples chosen for these experiments was set to ten since this was a convenient number of samples to analyze with the original method. To make sure that ten samples were enough, a student t-test was performed based on the standard deviation from previous experiments with Mepitel Ag. The power was set to 0.95 during the t-test. The acceptance criterion for the equivalence test was that the confidence interval for the difference between the averages should be less than one eight of the specification interval for Mepitel Ag.

For the current method, the silicone dressing was carefully removed and placed in a Digitube together with 50 µl of yttrium (1000 mg/l, 0.05 mg), 250 µl of indium (10000 mg/l2.5 mg) and 2 ml of DBSA. The samples were heated for 1 hour in 150° C. 6 ml of 32 % HCl was added and the samples were heated a second time for 10 min at 60° C. An additional 12 ml of 32 % HCl was added and the samples were shaken for 90 seconds. The organic phase together with most of the sample was removed and 3 ml was left. 10 % HCl was added to the samples for a total volume of 50 ml. Before analysis the samples were filtered with Digifilters and a filter manifold. The calibration curve used contained 0.003 mg, 0.013 mg, 0.055 mg, 0.235 mg and 1 mg of Ag in 10 % HCl. The samples were analyzed at wavelength 338.289 nm and the comparison of results can be seen in figure 31.

For the original method, the samples were removed the same way as in the current method and placed in acid rinsed beakers. 2 ml of $H_2SO_4$ was added to the beakers and heated on a heating plate for 1 hour, followed by 30 ml of $HNO_3$ and an additional 30 minutes of heating. The heating plate was turned off and 50 ml of water was added to the samples which were left for 10 minutes. The samples were transferred to acid rinsed 500 ml volumetric flasks and diluted with water up to the mark. Before analysis the samples was transferred to 15 ml falcon tubes. The samples were analyzed at wavelength 243.779 nm. The result is shown in figure 31 and can be compared to the product specification of 1.8-3.0 mg Ag/cm$^2$ of silver in Mepitel Ag.

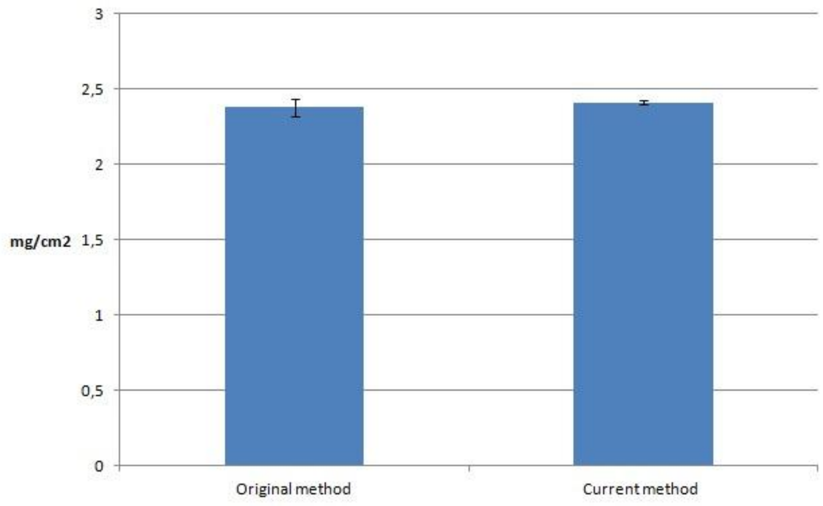

**Figure 31. Amount of silver in Mepitel Ag for comparison between the original method and the current method. n = 10, error bars show standard deviation.**

The difference between the method averages was tested with a student t-test and they complied with the acceptance criterion. The result shows insignificant difference between the methods.

In addition to Mepitel Ag, the current method was tested on two other products; Mepitel Film IV AM, a silver containing soft silicone adhesive dressing, and silver containing silicone earbuds from Steritech. For Mepitel film IV AM, the silicone was removed as described for Mepitel Ag. Two different parts of the earbuds was used, the outer part and the inner part. Both products were digested and analyzed as described above for method comparison. The results for the earbuds were below LOD. For Mepitel Film IV AM the silver content was determined to be 0.44 weight % silver sulfate (n = 3), which is in agreement with the specification of 0.5 weight %.

# 4. Discussions

## 4.1 Internal standard

When choosing a suitable internal standard it is possible to obtain information about interfering lines through literature. During this work each internal standard was also analyzed to evaluate possible effects and interferences with silver and the chosen matrixes. Even though some of the internal standards had interferences, the amount of IS that is added to the sample is important. Yttrium for example was added in a large amount in the initial experiments since the final IS volume was not yet determined. The large amount might be a reason why it showed a peak at 328.068 nm. The intensity of the IS signal is an important aspect as well, a low intensity might be diluted below LOD during samples preparation.

Internal standard used as a volume marker showed excellent results (section 3.2.2) and it was possible to detect a distinct difference between samples used with and without IS. The difference is mainly due to the loss of samples during filtration but an IS will compensate for this.

The use of IS to eliminate system variations was not optimal but the results was better with than without IS. To evaluate the possibility of eliminating system variations, DOE was chosen instead of COST. A COST evaluation would have been time consuming and may not have resulted in the right conclusions (Eriksson, et al., 2008). The design chosen for DOE was the CCP where the extreme values for each parameter was evaluated. There are more designs than CCP, for example Box-Behnken where the middle value of the chosen extremes are evaluated. This design is however not a factorial (U.S. Deparment of Commerce, 2013). During the evaluation only the nebulizer- and auxiliary gas was varied due to limitations in the ICP-OES. The values chosen for DOE might have been to extreme and should have been narrower in order to represent realistic changes in the parameters. At the beginning the nebulizer gas was set to vary between 0.1 and 0.3 ml/min but it was discovered that the sample aerosol did not reach the plasma at 0.1 ml/min. For a more thorough investigation, the peristaltic pump rate could have been changed and also the magnetic field around the torch in order to change the temperature of the plasma. Addition of calcium or cesium could also have been added to change the plasma temperature (Axelsson, 2014). The effect of tube size on the result could have been investigated as well. In order to investigate the robustness of the plasma and to investigate if the default settings were good, the ratio of the atomic and ionic wavelengths of magnesium can be used. A value higher than ten indicates a robust plasma (Sert, 2013).

The final method contains two phases, one oil phase consisting of DBSA and one water phase consisting of HCl. The desire is to extract both the silver and internal standard from the oil phase to the water phase. The internal standard and silver must have chemical and physical similarities to obtain this and there is a possibility that yttrium was not as compatible as first expected. An idea was that yttrium might have a higher affinity to oil compared to silver (figure 19). However, according to literature yttrium have a higher solubility towards water than oil (Bornstein, et al., 1997). Another possibility is that yttrium will bind to the sulfonate ions in the organic phase and form a complex; this might be a reason for the varying accuracies obtained for yttrium.

During the study of suitable IS other alternatives than indium, yttrium, lanthanum and rhodium were looked at, e.g. iridium. However, due to the economic aspect and time limitations it was not an alternative.

The economic aspect of internal standards was not the only limitation. The iTEVA computer software used during analysis could only analyze the results from two IS at once, therefore only two IS were chosen for further evaluation. During the second half of the thesis work, the software Qtegra that could evaluate several IS was installed. However, at that time indium and yttrium had already been chosen. Qtegra was more flexible to work with and the time for method development could have been reduced with this software installed from the beginning.

## 4.2 Method development

During method development it is essential to have properly cured silicone samples with encapsulated silver to obtain control samples as similar to the real products as possible. It would have been easier to add silver solution straight to pre-cured silicone samples, but then it would not have been possible to evaluate the digestion and release of silicone-encapsulated silver.

Swelling of samples was investigated at the beginning of the method development to allow a more thorough digestion of cross-linked silicone. The first idea was to use two separate substances for digestion, one that would swell the sample and then an acid for digestion. HMDS was the first option as swelling agent since it is similar to silicone (Lindgren, 2014). However during the investigation of swelling, DBSA was tested as a combined swelling agent and a digestion acid. DBSA is used as a catalyst during cross-linking and also hydrolysis and condensation of alkoxysilanes (Lindgren, 2014) (Yabuta, et al., 1996). A reaction with alkoxysilanes where the proton is donated by DBSA is seen in figure 32.

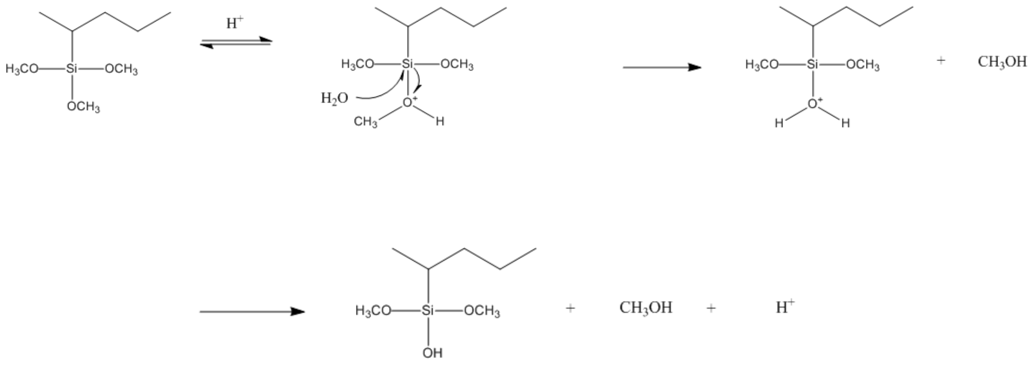

Figure 32. Hydrolysis reaction of alkoxysilanes.

A hydrolysis reaction can possibly be used to explain the digestion of silicone where DBSA donates a proton and hydrolysis the Si-O-Si bonds in the main silicone chain and form silanol groups at the ends (figure 33) (Lindgren, 2014). This reaction seems possible since DBSA can act as a Brønsted acid and donate a proton (Bigdeli, et al., 2011). The hydrophobic DBSA is believed to reach the siloxane bonds more effectively than a water based acid. However, there is a possibility for the created silanol groups to condense and form siloxane once again.

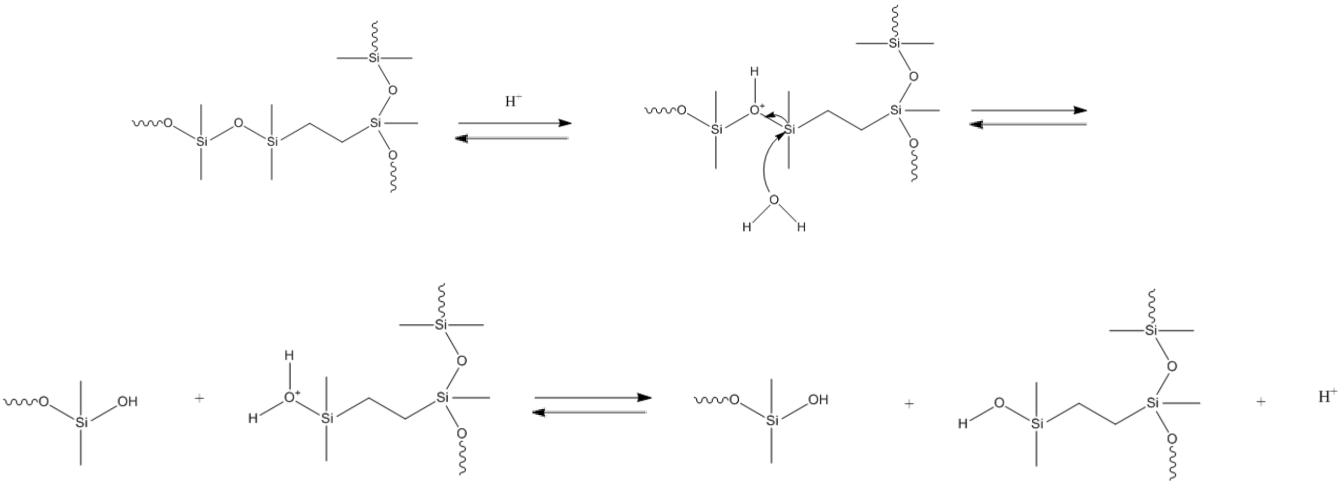

Figure 33. Hydrolysis of silicone with DBSA and the formation of silanol.

The metallic phase formed on the surface of the upper organic phase is probably silver in either metallic or ion form. The reason silver is suspected is due to a correlation detected during method development. It was possible to see an increase of the metallic phase when the amount of silver increased in the samples. For metallic silver to be created, a redox reaction needs to happen, however it is not known which one.

In the current method, the acid used for digestion is HCl. With $HNO_3$, it would have been possible to obtain a broader calibration range due to a higher solubility of silver (Gaines, n.d.). A wider ranger could eliminate the dilution step that is performed during the samples preparation step. Samples containing $HNO_3$ is less photosensitive and the risk of precipitation is lower. $HNO_3$ is however expected to nitrate DBSA and create potentially reactive mixture but this was not evaluated.

During the method development, shaking of samples were evaluated and 90 seconds were first chosen to make sure that all analytes came into contact with HCl. The 90 seconds were then reduced to 30 seconds, however it may even be enough with 10 seconds. Another stage that could have been evaluated is the one hour heating step. After 30 min it was possible to detect a homogenous phase where all sample was digested and probably all silver had already after this time been released.

To eliminate the acid-rinsing step, plastic Digitubes were used. When the tubes are heated in 150° C there is a risk of releasing of additives from the plastic, however this is not a problem during ICP-OES analysis. There is also a possibility of deformation of the plastic tubes during heating that will change the volume grading on the tubes, leading to an error during dilution. However, IS will act as a volume marker and variations in volume would not affect the results.

The use of microwave showed promising results but would require more time for rinsing the bombs before and after sample preparation. Due to these cleaning steps, open vessel digestion is a preferable alternative and is less time consuming. Microwave digestion is also limiting in number of samples digested each run compared to open vessel digestion. There is also a limitation in the choice of digestion acid. Since sulfonic acids are not possible to use, DBSA might not be possible either and therefore this current method can’t be used with microwave digestion. However, in a sample preparation method containing HF a microwave is to prefer due to a reduced exposure of operators to the acid.

## 4.3 Method validation

The method validation was done according to MHC standards. In this validation three replicates were made for each level of silver; low, middle and high. The validation will be performed by three persons to evaluate the reproducibility of the method. However, this has not yet been done but the obtained results indicate that this should not be a problem.

During the validation, a calibration curve was prepared in silicone to eliminate matrix differences between the curve and samples. This lead to a better result but will add some extra time to the samples preparation.

The samples were evaluated for stability for 7 days. More than seven days were not needed since the samples will be analyzed before that. However, the stability of the calibration curve could have been further evaluated to investigate the possibility to keep the curve for a longer time and for use in other analyzes. This can reduce the preparation time since there is no need to prepare a new calibration curve for each analysis.

## 4.4 Product testing

The product testing was not done according to the validated method, however the additional heating step that was performed and the longer time of shaking have been proven not to affect the results. The calibration curve used during product testing contained 0.01 mg of silver at the lowest level, a value that has shown a large variation in accuracy and is unreliable. However, the results for Mepitel Film IV AM was in the upper part of the calibration curve and would not be affected. The amount of silver in the earbuds was below LOD and could not be measured. However, if the analysis was performed with ICP-MS it may have been possible to detect the silver. ICP-MS has a higher sensitivity and can measure analytes down to ng/kg (Perkin Elmer, u.d.). The comparison between the original method and current method showed an insignificant difference.

## 5. Conclusions

A novel method was developed for determination of silver in silicone dressing. Dodecylbenzene sulfonic acid was used to digest the silicone dressing and HCl was used to extract the silver. A major improvement in the current method is the use of indium as internal standard which allows variations in sample preparation volume without affecting the results. Indium was not as optimal as first expected for elimination of system variations in the ICP-OES, but the result was significantly improved compared to not using it. The use of one vessel (Digitube) both for sample preparation and injection to the ICP-OES increases the through-put time which can have a large impact on e.g. product development. In addition, the developed method uses less acid during sample preparation and is performed in a heating block. This reduces the cost of analysis and improves the safety of the operators.

The method was validated according to MHC standards and the comparison between the original method and current method showed insignificant differences. Thus the developed method can be used for future analysis of silver in silicone dressing.

# 6. Future work

The final method is fast and shows excellent results; however it is possible to make it even more efficient. The stability time of the calibration curve can be evaluated in order to store it for other analysis. $HNO_3$ instead of HCl or a combination as extraction acid can also be investigated.

Further investigations can be done to find the most suiting internal standard to eliminate variations in the ICP system and to get a more thorough knowledge about ICP-OES.

In addition, evaluating the possibility of using the new method for determination of other metals than silver will aid MHC in their R&D work.

## 7. Acknowledgements

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- Kristina Hamberg for contribution of literature about silver.
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- Annelie Angerfors, my opponent

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# Appendix

The table contains the different variations of system variations evaluated during DOE at wavelength 243.779 nm.

| No. | Neb. gas [ml/min] | Aux. gas [l/min] | Silver conc. [mg/l] | Matrix | Internal standard | Accuracy [%] |
|---|---|---|---|---|---|---|
| **1** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | Yttrium | 137.6 |
| **2** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | Yttrium | 80.3 |
| **3** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | Yttrium | 187.7 |
| **4** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | Yttrium | 92.8 |
| **5** | 0.14 | 0 | 10 | 10 % $HNO_3$ | Yttrium | 135.6 |
| **6** | 0.3 | 0 | 10 | 10 % $HNO_3$ | Yttrium | 83.5 |
| **7** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | Yttrium | 129.8 |
| **8** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | Yttrium | 98.7 |
| **9** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | Yttrium | 136.9 |
| **10** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | Yttrium | 82.6 |
| **11** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | Yttrium | 132.8 |
| **12** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | Yttrium | 99.1 |
| **13** | 0.14 | 0 | 10 | 15 % $HNO_3$ | Yttrium | 136.5 |
| **14** | 0.3 | 0 | 10 | 15 % $HNO_3$ | Yttrium | 84.4 |
| **15** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | Yttrium | 129.9 |
| **16** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | Yttrium | 99.1 |
| **17** | 0.14 | 0 | 0.4 | Aqua regia | Yttrium | 163.1 |
| **18** | 0.3 | 0 | 0.4 | Aqua regia | Yttrium | 90.3 |
| **19** | 0.14 | 1.5 | 0.4 | Aqua regia | Yttrium | 146.9 |
| **20** | 0.3 | 1.5 | 0.4 | Aqua regia | Yttrium | 92.9 |
| **21** | 0.14 | 0 | 10 | Aqua regia | Yttrium | 140.6 |
| **22** | 0.3 | 0 | 10 | Aqua regia | Yttrium | 84 |
| **23** | 0.14 | 1.5 | 10 | Aqua regia | Yttrium | 131.7 |
| **24** | 0.3 | 1.5 | 10 | Aqua regia | Yttrium | 92.1 |
| **25** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | Indium | 144.4 |
| **26** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | Indium | 65.2 |
| **27** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | Indium | 195.3 |
| **28** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | Indium | 51.8 |
| **29** | 0.14 | 0 | 10 | 10 % $HNO_3$ | Indium | 147 |
| **30** | 0.3 | 0 | 10 | 10 % $HNO_3$ | Indium | 67.9 |
| **31** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | Indium | 138.3 |
| **32** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | Indium | 54.9 |
| **33** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | Indium | 148.4 |
| **34** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | Indium | 67.5 |
| **35** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | Indium | 142.9 |
| **36** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | Indium | 55.1 |
| **37** | 0.14 | 0 | 10 | 15 % $HNO_3$ | Indium | 145.6 |
| **38** | 0.3 | 0 | 10 | 15 % $HNO_3$ | Indium | 68.7 |
| **39** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | Indium | 137.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **40** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | Indium | 53.8 |
| **41** | 0.14 | 0 | 0.4 | Aqua regia | Indium | 183.5 |
| **42** | 0.3 | 0 | 0.4 | Aqua regia | Indium | 77.4 |
| **43** | 0.14 | 1.5 | 0.4 | Aqua regia | Indium | 157.2 |
| **44** | 0.3 | 1.5 | 0.4 | Aqua regia | Indium | 54.9 |
| **45** | 0.14 | 0 | 10 | Aqua regia | Indium | 159.5 |
| **46** | 0.3 | 0 | 10 | Aqua regia | Indium | 70.9 |
| **47** | 0.14 | 1.5 | 10 | Aqua regia | Indium | 140.6 |
| **48** | 0.3 | 1.5 | 10 | Aqua regia | Indium | 54.5 |
| **49** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | No IS | 172.1 |
| **50** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | No IS | 58.5 |
| **51** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | No IS | 116.6 |
| **52** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | No IS | 19.5 |
| **53** | 0.14 | 0 | 10 | 10 % $HNO_3$ | No IS | 174.7 |
| **54** | 0.3 | 0 | 10 | 10 % $HNO_3$ | No IS | 61.7 |
| **55** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | No IS | 115.3 |
| **56** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | No IS | 22.6 |
| **57** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | No IS | 173.4 |
| **58** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | No IS | 60 |
| **59** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | No IS | 117.3 |
| **60** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | No IS | 20.9 |
| **61** | 0.14 | 0 | 10 | 15 % $HNO_3$ | No IS | 165.8 |
| **62** | 0.3 | 0 | 10 | 15 % $HNO_3$ | No IS | 63 |
| **63** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | No IS | 114.5 |
| **64** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | No IS | 21.8 |
| **65** | 0.14 | 0 | 0.4 | Aqua regia | No IS | 213.8 |
| **66** | 0.3 | 0 | 0.4 | Aqua regia | No IS | 67 |
| **67** | 0.14 | 1.5 | 0.4 | Aqua regia | No IS | 125.2 |
| **68** | 0.3 | 1.5 | 0.4 | Aqua regia | No IS | 21.3 |
| **69** | 0.14 | 0 | 10 | Aqua regia | No IS | 185.5 |
| **70** | 0.3 | 0 | 10 | Aqua regia | No IS | 60.2 |
| **71** | 0.14 | 1.5 | 10 | Aqua regia | No IS | 112 |
| **72** | 0.3 | 1.5 | 10 | Aqua regia | No IS | 22.5 |
| **73** | 0.22 | 1 | 5.2 | 10 % $HNO_3$ | Yttrium | 94.6 |
| **74** | 0.22 | 1 | 5.2 | 10 % $HNO_3$ | Yttrium | 96.6 |
| **75** | 0.22 | 1 | 5.2 | 10 % $HNO_3$ | Yttrium | 97.9 |

The table contains the different variations of system variations evaluated during DOE at wavelength 338.289 nm.

| No. | Neb. gas [ml/min] | Aux. gas [l/min] | Silver conc. [mg/l] | Matrix | Internal standard | Accuracy [%] |
|---|---|---|---|---|---|---|
| **1** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | Yttrium | 103.9 |
| **2** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | Yttrium | 94.6 |
| **3** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | Yttrium | 116.5 |
| **4** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | Yttrium | 104.6 |
| **5** | 0.14 | 0 | 10 | 10 % $HNO_3$ | Yttrium | 104.2 |
| **6** | 0.3 | 0 | 10 | 10 % $HNO_3$ | Yttrium | 91.8 |
| **7** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | Yttrium | 105.8 |
| **8** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | Yttrium | 96.9 |
| **9** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | Yttrium | 101.7 |
| **10** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | Yttrium | 92.3 |
| **11** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | Yttrium | 104.9 |
| **12** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | Yttrium | 98 |
| **13** | 0.14 | 0 | 10 | 15 % $HNO_3$ | Yttrium | 104.5 |
| **14** | 0.3 | 0 | 10 | 15 % $HNO_3$ | Yttrium | 93.9 |
| **15** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | Yttrium | 105.5 |
| **16** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | Yttrium | 97.3 |
| **17** | 0.14 | 0 | 0.4 | Aqua regia | Yttrium | 114.7 |
| **18** | 0.3 | 0 | 0.4 | Aqua regia | Yttrium | 104.6 |
| **19** | 0.14 | 1.5 | 0.4 | Aqua regia | Yttrium | 116.2 |
| **20** | 0.3 | 1.5 | 0.4 | Aqua regia | Yttrium | 105.5 |
| **21** | 0.14 | 0 | 10 | Aqua regia | Yttrium | 111.3 |
| **22** | 0.3 | 0 | 10 | Aqua regia | Yttrium | 98 |
| **23** | 0.14 | 1.5 | 10 | Aqua regia | Yttrium | 108.9 |
| **24** | 0.3 | 1.5 | 10 | Aqua regia | Yttrium | 97.6 |
| **25** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | Indium | 64.6 |
| **26** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | Indium | 102.8 |
| **27** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | Indium | 106 |
| **28** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | Indium | 93.8 |
| **29** | 0.14 | 0 | 10 | 10 % $HNO_3$ | Indium | 67 |
| **30** | 0.3 | 0 | 10 | 10 % $HNO_3$ | Indium | 103.9 |
| **31** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | Indium | 99.2 |
| **32** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | Indium | 90 |
| **33** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | Indium | 64.5 |
| **34** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | Indium | 105.6 |
| **35** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | Indium | 98.6 |
| **36** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | Indium | 92.3 |
| **37** | 0.14 | 0 | 10 | 15 % $HNO_3$ | Indium | 69 |
| **38** | 0.3 | 0 | 10 | 15 % $HNO_3$ | Indium | 105.9 |
| **39** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | Indium | 99.4 |
| **40** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | Indium | 91.9 |
| **41** | 0.14 | 0 | 0.4 | Aqua regia | Indium | 80.5 |
| **42** | 0.3 | 0 | 0.4 | Aqua regia | Indium | 106.1 |
| **43** | 0.14 | 1.5 | 0.4 | Aqua regia | Indium | 104.2 |

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|---|---|---|---|---|---|---|
| **44** | 0.3 | 1.5 | 0.4 | Aqua regia | Indium | 91.2 |
| **45** | 0.14 | 0 | 10 | Aqua regia | Indium | 79.9 |
| **46** | 0.3 | 0 | 10 | Aqua regia | Indium | 100.7 |
| **47** | 0.14 | 1.5 | 10 | Aqua regia | Indium | 99.5 |
| **48** | 0.3 | 1.5 | 10 | Aqua regia | Indium | 85.1 |
| **49** | 0.14 | 0 | 0.4 | 10 % $HNO_3$ | No IS | 112.3 |
| **50** | 0.3 | 0 | 0.4 | 10 % $HNO_3$ | No IS | 93 |
| **51** | 0.14 | 1.5 | 0.4 | 10 % $HNO_3$ | No IS | 95 |
| **52** | 0.3 | 1.5 | 0.4 | 10 % $HNO_3$ | No IS | 46.6 |
| **53** | 0.14 | 0 | 10 | 10 % $HNO_3$ | No IS | 112.3 |
| **54** | 0.3 | 0 | 10 | 10 % $HNO_3$ | No IS | 89.5 |
| **55** | 0.14 | 1.5 | 10 | 10 % $HNO_3$ | No IS | 86 |
| **56** | 0.3 | 1.5 | 10 | 10 % $HNO_3$ | No IS | 42.3 |
| **57** | 0.14 | 0 | 0.4 | 15 % $HNO_3$ | No IS | 107.9 |
| **58** | 0.3 | 0 | 0.4 | 15 % $HNO_3$ | No IS | 92.9 |
| **59** | 0.14 | 1.5 | 0.4 | 15 % $HNO_3$ | No IS | 85 |
| **60** | 0.3 | 1.5 | 0.4 | 15 % $HNO_3$ | No IS | 42.8 |
| **61** | 0.14 | 0 | 10 | 15 % $HNO_3$ | No IS | 115.3 |
| **62** | 0.3 | 0 | 10 | 15 % $HNO_3$ | No IS | 90.9 |
| **63** | 0.14 | 1.5 | 10 | 15 % $HNO_3$ | No IS | 85.2 |
| **64** | 0.3 | 1.5 | 10 | 15 % $HNO_3$ | No IS | 43.7 |
| **65** | 0.14 | 0 | 0.4 | Aqua regia | No IS | 129.4 |
| **66** | 0.3 | 0 | 0.4 | Aqua regia | No IS | 100.8 |
| **67** | 0.14 | 1.5 | 0.4 | Aqua regia | No IS | 92.8 |
| **68** | 0.3 | 1.5 | 0.4 | Aqua regia | No IS | 48.8 |
| **69** | 0.14 | 0 | 10 | Aqua regia | No IS | 126.3 |
| **70** | 0.3 | 0 | 10 | Aqua regia | No IS | 94.2 |
| **71** | 0.14 | 1.5 | 10 | Aqua regia | No IS | 81.1 |
| **72** | 0.3 | 1.5 | 10 | Aqua regia | No IS | 45.2 |
| **73** | 0.22 | 0.75 | 5.2 | 10 % $HNO_3$ | Yttrium | 83.5 |
| **74** | 0.22 | 0.75 | 5.2 | 10 % $HNO_3$ | Yttrium | 85.1 |
| **75** | 0.22 | 0.75 | 5.2 | 10 % $HNO_3$ | Yttrium | 85 |