Determination of the silver concentration in different plant parts

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Date: 4 August 2016

Summary

Intra Hydrocare is widely used in greenhouses to clean and disinfect water systems. Intra Hydrocare consists of the actives hydrogen peroxide and chelated silver. The combination has a unique synergistic effect. In this study it was established that the silver in Intra Hydrocare does not accumulate in plant parts (roots, stem and leaves) and not in the substrate. A small increase in the silver concentration was found in the roots, the increase was in PPB level. What this means and how this can protect the plant against pathogenic invaders like Crazy roots will be further investigated. The study was performed at the laboratory of Intracare.

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Introduction

Intra Hydrocare is based on a synergistic mix between hydrogen peroxide (HP) and silver (Ag). In this study it was investigated if there is any buildup of silver in different plant parts. Intra Hydrocare consist of 590 g/l hydrogen peroxide (HP) and 0,36 g/l silver (Ag).

Hydrogen peroxide

Hydrogen peroxide is a clear, colourless and odourless liquid which is miscible with water in all ratios. It is a simple chemical compound which is composed of one water molecule (H_2O) and one oxygen atom (O). HP only differs from water by this additional oxygen atom. The chemical formula of HP is H-O-O-H or H_2O_2.

The formula clearly indicates that the 2 oxygen atoms are directly bound with each other, namely -O-O-. Such combination of 2 oxygen atoms is called 'peroxide group'. The peculiarity of this peroxide group is that one of both oxygen atoms relatively easy transfers -via all sorts of intermediate reactions - to other molecules or groups. This process is called 'oxidation' and the transferred oxygen atom is named 'active oxygen'. HP is the simplest of the active oxygen compounds.

In contact with impurities of all kinds, like metals and their salts, alkali and UV-radiation HP becomes 'activated' and the H_2O_2 decomposes very rapidly. During this activation or fastened decomposition all sorts of highly disinfective active oxygen compounds are released. At the end of the reaction the HP is completely decomposed in water and oxygen.

$H_2O_2 \rightarrow$ active oxygen $\rightarrow$ oxidation $\rightarrow$ disinfection $\rightarrow 2\ H_2O + O_2$

HP can be activated by certain metals and their salts, UV-radiation, alkali,… what results in a fastened decomposition of hydrogen peroxide. During this activated decomposition one of the oxygen atoms (active oxygen) is removed from the peroxide group. This reaction forms different types of intermediary products like; hydroxyl radicals (•OH), peroxyl radicals (•O-O-H) and peroxyl anions ($^{-}$O-O-H). All these oxygen compounds have excellent disinfective properties and are classified under one name, the 'active oxygen compounds'.

Silver

The disinfectant properties of silver have been known for centuries (Laubusch 1971). History is full of examples in which Ag has been used for its purification properties. According to Russell (1994), Aristotle advised Alexander the Great to boil water and store it in Ag vessels to prevent waterborne diseases.

Pioneers that crossed America placed silver coins in their water barrels. Vikings would line the hull of their ships with strings of silver and copper to prevent growth of algae and barnacles. Notably, modern ships still use silver and copper for the same purpose (Laubusch 1971). Although most early civilizations did not fully comprehend the antibacterial properties of silver, it was widely understood that adding Ag to water increased clarity, reduced odor and improved taste. Until recently, silver water purification techniques had fallen out of favor for more fast acting methods such as chlorination.

Silver electrochemistry methods were reexamined in the 1960s when NASA developed an electrolytic Ag ionizer to purify drinking water in the Apollo spacecraft. Today Ag is used to prevent infections in burn patients, to prevent blindness in newborns, to make bacteria-free cosmetics, to disinfect water storage containers including swimming pools, to control Legionella bacteria in hospitals and to improve the performance of drinking water filters.

Ag is a particularly effective bacteriostatic agent (prevents the growth or reproduction of bacteria). Ag ions use three main mechanisms to control bacterial growth.

a) Remove hydrogen atoms from sulfhydryl groups (-SH) on bacteria and viruses.
b) Inhibit DNA replication by interfering with DNA unwinding.
c) Alter the bacterial membrane with enzyme mechanisms.

Hydrogen Peroxide and Silver

Multiple theories had been proposed for the biological and chemical toxic mechanisms of Ag and HP (Thurman & Gerba 1989, Pedahzur et al 2000). Further microbiological research is required to validate any one of the theories. However, there are some common understandings. The common theories can be considered in a chemical and biological context. Pedahzur et al (1997) assessed the chemical context of HP and Ag toxicity. Three chemical interactions were observed: stabilization of HP by silver to limit HP breakdown; interference of HP in silver efflux from cell walls; and interference of Ag with HP cellular detoxification.

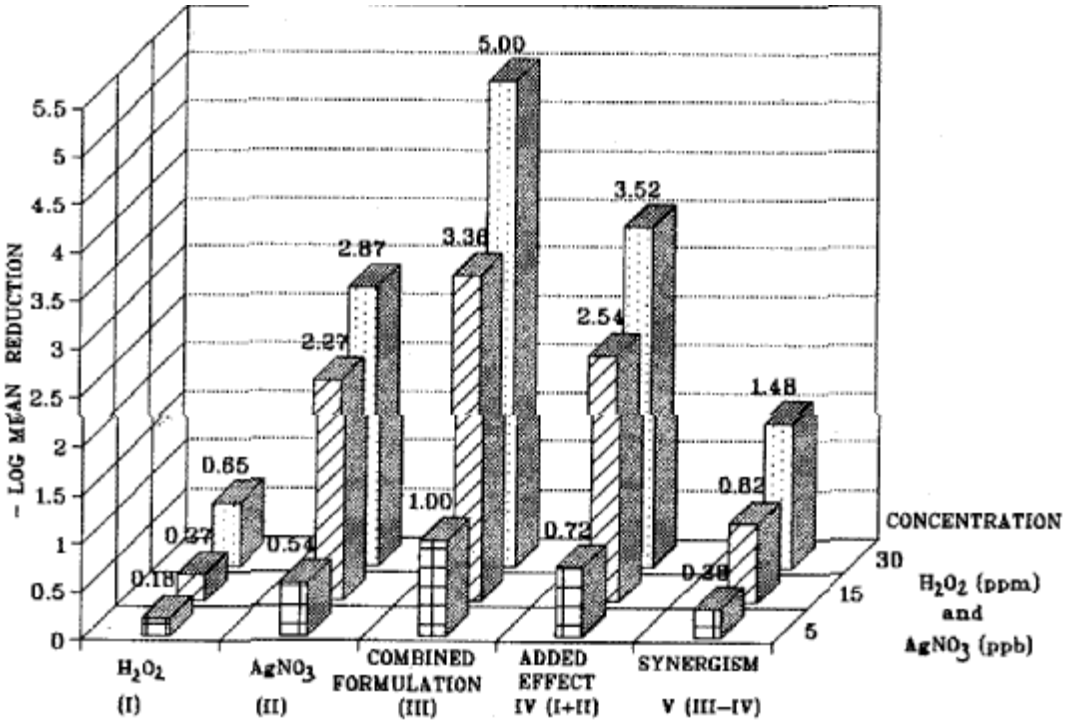
Therman and Gerba (1989) studied the biological effects of the HP/Ag system. It was found that Ag and HP interfere with electron transport, DNA replication, and the cell walls of viruses and bacteria. Ag has a particularly high affinity for sulfhydryl groups on bacterial cell membranes. By binding to sulfhydryl groups on cell walls, bacterial respiration is prevented. The synergistic results indicate that the toxic processes for Ag and HP are metabolically related and potentiate similar responses (Pedahzur et al 2000).

The chemical relation between H_2O_2 and silver and microorganisms?

In a study to the synergistic effect of Ag and HP (Pedahzur, *et. al.* 1995) on the inactivation of *E. Coli* it was found that a combination of silver and hydrogen peroxide has a higher inactivation performance than the sum of the inactivitation levels of the separate disinfectants. The bactericidal mode of action is that silver ions react with the cellular-SH groups, which are of importance in the activity of many enzymes and in protein structure. Silver is reported to inhibit dehydrogenation processes and phosphate uptake and also promote the efflux of phosphate, K+ ions and other substructures. Ag also interacts with nucleic acids.

The synergistic effect is that because HP and Ag have their individual mode of actions it is susceptible that an accumulation of cellular damages caused by the individual components or on agent rendering the cell more susceptible to the effects of the other. In a study it was demonstrated that silver and HP act synergistically on the viability of pathogenic cells.

In some cases, it was found that the combined system was between 100 and 1000 times more effective at controlling *E. coli* bacteria growth than the sum of the individual chemicals.



The chemical relation between silver and H2O2

HP is inherently unstable, when Intracare purchase HP the stability is maximum 5%. The stability is increased by adding the silver concentrate called Intra Argentum. In the silver concentrate there are different ingredients that increase the stability of the HP to a decomposition rate of < 1% a year. The production of Intra Argentum is proprietary knowledge of Intracare.

There are different components in Intra Argentum that contribute to the increased stability of Intra Hydrocare. Because the excellent stability of Intra Hydrocare we can guarantee a long storage live and safe transportation. Additionally its low rate of decomposition assure long lasting residual disinfection.

Mode of action of Ag and HP

The combination of Ag and HP gives improved inactivation performance (bacteriostatic) as compared with hydrogen peroxide and silver alone. While the primary HP targets are the lipids, proteins and nucleic acids it is suggested that Ag mainly acts on SH protein groups, inhibits DNA replication and alter the cell membrane. The enhancement of the HP's activity may be associated with the damage to the enzymes involved in the hydrogen peroxide deactivation.

Greenhouses

Intra Hydrocare is widely used to clean irrigation ecosystems and disinfect. Irrigation systems are a source of pathogenic fungi and bacteria growth which can adversely affect the yield of the tomato plant. Also make sure the bacteria in the water lines a constant formation of biofilm. Biofilm is a layer that is created by bacteria where they can settle and multiply, and sits on the inside of the pipe. As a biofilm in the course of time, growth of the biofilm can affect (decrease) the natural flow of the water. This results in an uneven growth in the greenhouse that comes at the expense of the homogeneity of the tomato.

Intra Hydrocare is a product that will degrade both the biofilm and destroy the pathogens and remove bacteria and fungi. The active substances are water-peroxide which acts as a powerful oxidizer, and silver chelated that acts as a supported bactericide and for stabilization.

Hydrogen peroxide reacts during the chemical conversion into oxygen and hydrogen peroxide, two harmless products.

Intra Hydrocare is administered continue in a 50 ppm concentration what is related to 25 ppm hydrogen peroxide and 1.8 ppm silver.

Method

A tomato grower located in the Netherlands was selected for this test. The test started at 3 February and will run during the whole lifecycle of the tomato plant. The concentration Intra Hydrocare that is continuously dosed is 40 ppm.

Data

Species:	Vine tomato
Start dosing Intra Hydrocare:	3 February 2016
Concentration Intra Hydrocare:	40 ppm, continue
Date harvesting plant material:	28 July 2016

The materials were separated according to the different plant parts: Leaf – Root – Stem – Rock Wooll

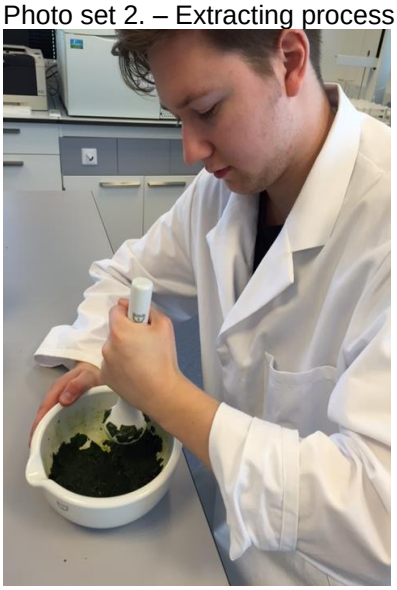
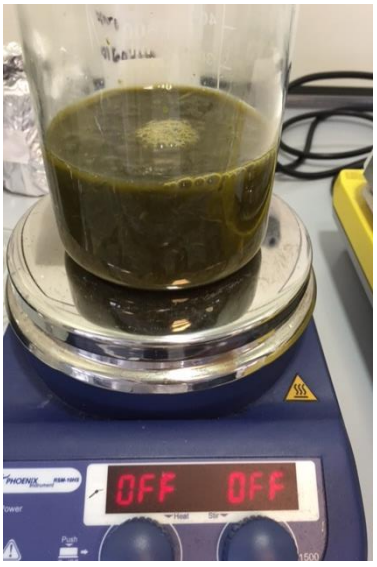
Photo set 1. Plant parts of the tomato plant



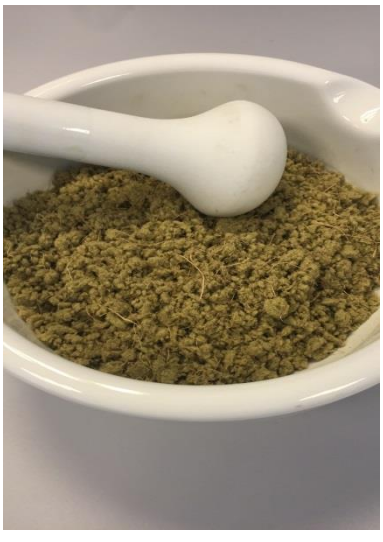

After the separation process, the homogenous parts were crushed with a mortar and a 2% nitric acid solution was added.

The whole mixture was boiled during two hours and it was filtered over a 4 um filter paper. The remaining filtrate solution was clear and measured with the AAS.

Photo set 2. – Extracting process of the plant materials






Atomic Absorption Spectrophotometer

The AAS or atomic absorption spectrophotometer is a method that can detect silver at low concentrations on ppb level (part per billion). The method is added in the annex. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law.

The electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law.

Figure 1. Atomic absorption spectrophotometer diagram

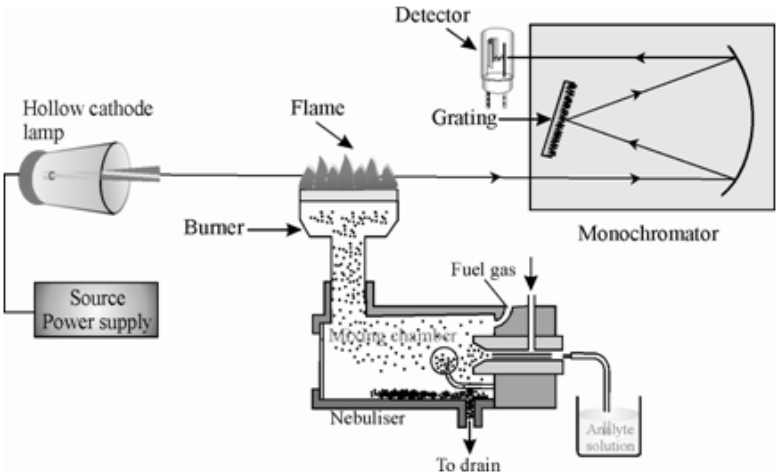


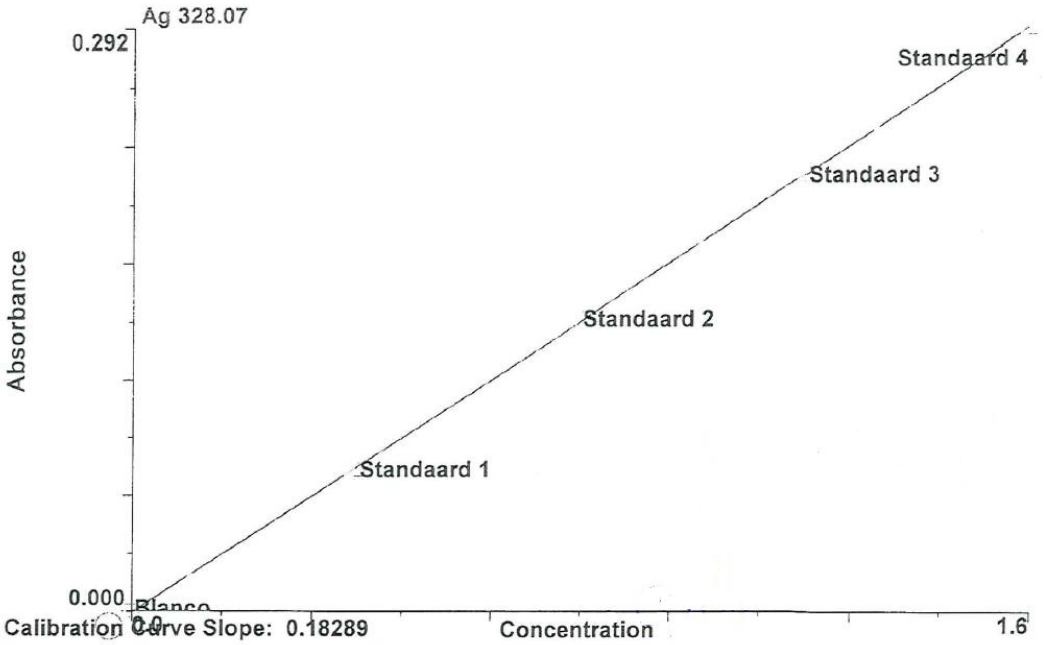
Photo 3. AAS at Intracare

Results

Silver Calibration line

Edit Calibration

Result: 03082016ag



Calibration Curve Slope: 0.18289
Calibration Curve Intercept: -0.00034
Calibration Curve Correlation Coefficient: 0.999958
Calibration Curve Type: Linear, Calculated Intercept
Current Sample Concentration: -0.040 mg/L

Calibration data for Ag 328.07 — Equation: Linear, Calculated Intercept

ID	Mean Signal (Abs)	Entered Conc. mg/L	Calculated Conc. mg/L	Standard Deviation	%RSD
Blanco	0.000	0	0.002	0.00	14.41
Standaard 1	0.071	0.4	0.392	0.00	0.71
Standaard 2	0.147	0.8	0.808	0.00	0.69
Standaard 3	0.219	1.2	1.200	0.00	0.37
Standaard 4	0.292	1.6	1.598	0.00	0.16

Correlation Coef.: 0.999958 Slope: 0.18289 Intercept: -0.00034

Equation of the calibration curve:

(1) $y = ax + b$

(2) $y = 0.18289x + (-0.040)$

Table 1. – Measurement results

Plant part	Amount (g)	Extraction volume (ml 2% HNO3)	Absorption (AU)
Leaf	67.5	200	0.005
Stem	151.5	400	0.005
Tomato	460	200	0.005
Root	27.7	200	0.011
Substrate	90.1	400	0.005
Control			0.005

The average background absorption is 0.005, this implicates that there is no significant absorption by silver in the plant parts Leaf, Stem, Tomato and substrate. When we look at the concentration of root we can see a rise in the absorption by 0.011 – 0.005 = 0.006 AU.

The concentration that is related to this concentration can be calculate:

y = 0.18289x + (-0.040)

0.006 = 0.18289x + (-0.040)

x = 0.2515 mg/l

x = 0.0002515 mg/ml

200 x 0.0002515 = 0.0503 mg

0.0503 / 90.1 = 0.00055826 mg/g

Conclusion

First of all this is the first test to evaluate the concentration of silver in plant part of tomato. It is important not to conclude to quick and wait for another measurement what is plant in November. On first side it can be concluded that there is a small rise in the silver concentration in the plant part root. What this means and if this can have a positive effect on the protection of the tomato plants has to be studied.

Annex Method Determination of silver with atomic absorption spectrofotometer

1. Scope

To determine the quantity of silver in Intra Hydrocare or Silver Concentrate with the Atomic Absorption Spectrometer.

2. Principle

Atomic emission spectrometry is a method for determining the concentration of an element in a substance by measuring the intensity of one of the emission lines of the atomic vapour of the element generated from the substance. The determination is carried out at the wavelength corresponding to this emission line.
Dissolution of the sample in water. Nebulization of the solution into an acetylene flame. Measurement of the absorption of the radiation at a wavelength, specific for the analyte, emitted by a hollow-cathode lamp. Correction for non-atomic absorption with a deuterium lamp.

3. Apparatus and Equipment

Atomic absorption spectrometer, Perkin Elmer SpectrAA 400, fitted with:

- Simultaneous background correction system.
- Hollow-cathode lamps for Silver elements.
- Spray chamber with mixing paddle.
- Adjustable nebulizer.
- Burner, suitable for a acetylene flame.
- Autosampler
- WinLab32AA Software

Further requirements

- Tubes, 10 ml fitted with a screw-cap, e.g. darkened polypropylene tubes, e.g. Roth
- Volumetric flask of 100.0 and 10.0 ml, amber glass bottles, e.g. Duran
- Piston Pipette, 1.00 – 5.00 ml, e.g. Roth No. 9092784.
- Piston Pipette, 100 – 1000 ul, e.g. Roth No. 9110101.

4. Reagents

- Silver standard solution
- Water: doubly distilled or of equivalent purity
- Concentrated nitric acid c=(HNO_3) 16 mol/l
- Acetylene, under pressure e.g. Airproducts, 10.00 L/min
- Air, under pressure e.g. Airproducts, 2.50 L/min
- Silver standard solution, *c*(Ag) = 1000 mg/l : Dilute a silver stock solution, *c*(Ag) = 1000 mg/l, e.g. Roth. 2349, 100 times with water pH 2 and homogenize.
- Water pH 2: Adjust the water to pH 2 with concentrated nitric acid c=(HNO_3) 16 mol/l.

5. Sample

Take a representative sample of Hydrocare or Silver Concentrate in a polyethylene flask.

6. Procedure

6.1 Apparatus settings

Method of analysis WinLab32 AA Software: Ag determination

Analyte	*Lamp current*	*Slit width*	*Wavelength*	*Flame*

	*mA**	*mm**	*nm*	
Silver	3	2.7	328.07	Air/C_2H_2

*: dependent on the apparatus

Background correction : switched off.
Delay time : 30 seconds.
Measurement time : 3 seconds.
Replicates : 3.
Acetylene : 10.00 L/min
Air : 2.50 L/min
Characteristic conc. : 0.02 mg/l = 0.0044 Au
Sensitivity check : 1.1 mg/l = 0.2 Au
Linear up to : 1.00 mg/l

6.2 Calibration solutions

Transfer to a series of four 25.0 ml volumetric flasks, using a 1000 – 5000 µl piston pipette, respectively 0, 1.00, 2.00, 3.00 and 4.00 ml of the silver standard solution. Protect the solution from light wrapping it up with aluminium foil. Dilute to volume with water pH 2 and homogenize. Pipette 1.0 ml of the analyte concentration to a 100.0 ml dark volumetric flasks, using a 100 – 1000 µl piston pipette, add to the level with water pH 2, and homogenize. The concentration of the calibration solutions are stated in the next Table:

Analyte	*K_o mg/l*	*K_1 mg/l*	*K_2 mg/l*	*K_3 mg/l*	*K_4 mg/l*
Silver	0	0.4	0.8	1.2	1.6

Calculate the calibration line: $y = bx + a$

The line parameters *b* and *a* are calculated with the following equations

$$b = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sum_i (x_i - \bar{x})^2}$$

and

$$a = \bar{y} - b\bar{x}$$

Calculate the correlation coefficient:

$$r = \frac{\sum_i ((x_i - \bar{x})(y_i - \bar{y}))}{\sqrt{\sum_i (x_i - \bar{x})^2 \sum_i (y_i - \bar{y})^2}}$$

where

x_1 = concentrations of standards

$\bar{x}$ = mean of concentrations of standards
y_1 = instrument response to standards
$\bar{y}$ = mean of instrument responses to standards

The calibration line is excepted when the correlation coefficient is > 0.995

QC sample
Use the Calibration Solution K2 as QC sample to check the linearity of the calibration line and to check drift of the baseline.

6.3 Test sample solution (in triplicate)

Transfer a test portion of x ml (= *Vs* ml or g) to a 100.0 ml volumetric flask, with water pH 2 and homogenize.

6.4 Measurement

Compose a test series according to the next Table:

Part	*Solutions to be measured*
1: calibration	K_0 K_1 K_2 K_3 K_4 K_{QC}
2: analysis	K_s K_{QC} n = 5 test sample solutions
3: end of test series	K_{QC}

Nebulize the solutions successively into the flame and determine the absorbance (= *A*) against water pH 2.

7. Expression of results

7.1 Calculation

- Have the software calculate the mean absorbance of the replicate measurements of the (blank) calibration solutions and from these values the ***calibration function***. Next calculate from this calibration function the mean results of the replicate measurements of all other solutions.
- Correct if necessary all mean results of the solutions measured between two consecutive pairs of blank calibration solutions (K_0 K_0 in parts 2 and 3 in the Table in 6.5) for ***baseline drift*** by interpolating with time between both second measurements of these blank calibration solutions (the first may be cross-contaminated!).
- For calculation of the concentration use the bracketing spreadsheet, thus correcting random errors or instrumental drift. With the bracketing the samples between the bracketing samples are corrected accordingly.
- Calculate the **analyte concentration in the solutions** by correcting all (eventually corrected for baseline drift) mean results of the solutions measured between two consecutive calibration solutions K_4 for **long-term drift** of the sensitivity with the (with time) interpolated recoveries of these two calibration solutions.
- Calculate the **analyte concentration**, [*c*(Ag)], **in the test sample** using the formula:

$$c(\mathrm{Ag}) = \frac{\bar{X}_s}{Vs \,.\, 1000} \,.\, 100 \quad (\mathrm{mg/ml})$$

where:

$\bar{X}_s$ = analyte concentration in the test sample solution, in mg/l;
Vs = mass of the test portion, in ml;

- Report the result to the nearest 0.1 %.

8. System Suitability

Measurements are always proceeded by a calibration with calibration standards, otherwise the analysis procedure will not work.

After installation of the copper lamp, the performance of the apparatus is checked before use and the results are registered in the log book:

- Check if the current is 15
- Check if the slit is 2.7/0.8
- Check if the energy level is 80
- Optimize the flame position by adjusting the burning chamber until maximal absorption
- Optimize the nebula chamber tightness until maximal absorption
- Optimize the gas mixture until maximal absorption
- Perform a repeatability test where one sample is analyzed 6 times. The RSD is not higher than 1%
- Maintenance is done yearly. Service report is filed in the 'Maintenance and Repair' folder.

9. Notes

- Cleaning glassware and flasks

Clean glassware, by rinsing with a 1.25% (v/v) ammonium solution first and with demineralised water at least 3x afterwards.

- Storage reagents

  Store all solutions in polyethylene or PTFE flasks to prevent contamination.

- Stability of solutions

  The certified stock solution and the element solutions may be stored for about one year.

- Pressure values and flow capacity

  See the manual of the instrument to be used for pressure values and flow capacity.

- Apparatus settings

  Before starting measurements the settings of the equipment should be optimized.

- Number of (blank) calibration solutions and test sample solution

  The number of blank calibration solutions and check calibration solutions to be used depends on the number of test sample solutions to be measured. The total number of test sample solutions (n) measured between two consecutive control calibration solutions (K_4) should be ≤ 10.

- Precision

  The precision is strongly dependent on the apparatus used and the experimental conditi-ons. Prevention of contamination is of great importance.

10. References

- Normative references
- NPR 6416
- Atomaire-absorptiespectrometrie. Vlamtechniek. Algemene richtlijnen.
- Atomic absorption spectrometry
- Ph Eur method 2.2.22