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# **ACKNOWLEDGEMENT**

The internship opportunity I had with African Technical Research Centre (ATRC) was a great chance for learning and professional development. Therefore, I consider myself as a very lucky individual as I was provided with an opportunity to be a part of it. I am also grateful for having a chance to meet so many wonderful people and professionals who led me though this internship period.

Bearing in mind previous I am using this opportunity to express my deepest gratitude and special thanks to the Director of ATRC Dr. Johnson Ouma, Head of analytical unit Mr. Wycleff Karanja, Assistant analytical chemist Mr. Festo Nicas, lab technicians Ms. Glory Martin and Ms. Grace Phaison who in spite of being extraordinarily busy with their duties, took time out to hear, guide and keep me on the correct path and allowing me to carry out my activities at their esteemed organization and extending during the training.

# **ABSTRACT**

Included in this report are accounts of my three months internship at ATRC-VHI. The report shows the overview of what was learnt in analytical unit during the whole period of the internship in details with the outstanding help from my supervisors Mr. Wycleff Karanja, Mr. Festo Nicas, Madam Glory Martin and Madam Grace Phaison. This internship has provided me with the real-world experience that which has enabled me to put everything I learned into action. Being at ATRC-VHI as an intern has given me the chance to see what the work setting looks like and the experience and skills has obtained has really given me a strong solid foundation of my career. Everything learnt during the whole period of internship can be summarized as follows; Sample preparation & extraction and Operation of HPLC, GC, Spectrophotometer and other laboratory equipment, implementation and auditing of ISO 9001:2015 and GLP. All of the learned activities are explained in this report in different sections which are; INTRODUCTION, METHODOLOGY AND DISCUSSION.

# **INTRODUCTION**

ATRC is a private R&D center owned by VHI. The research center is an expansion of a business relationship between AtoZ and Sumitomo Chemical Company. ATRC has been in operation since June 2012 and focuses on R&D for innovation of new agricultural, public health, vector and consumer products. Since its inception, the center has hosted two sitting presidents, HE President DR. Jakaya kikwete the President of the United Republic of Tanzania and HE President Ellen Johnson-Sirleaf of Liberia. ATRC is headed by the director who reports to the president of the joint venture company, ATRC houses three core functional units: Vector Control; Agronomy; and Analytical Chemistry unit. Each of the unit is headed by a senior scientist supported by a team of junior scientists and technicians.

## ACTIVITIES CARRIED OUT BY THE ANALYTICAL UNIT

The analytical unit carries two major activities;

### Analysis of chemical and physical parameters of lab samples.

### Analytical method development.

#### Analysis of physical and chemical parameters of net samples

The physical parameters analyzed by the analytical unit include; Gram per meter square (GSM), Dimension Stability, Bursting Strength, Mesh Size and the chemical parameters includes determination of an active ingredient of the lab samples at different harsh conditions such as CIPAC wash and at accelerated conditions and determination. During my internship there were two types of net samples analyzed by the analytical unit and each type of net samples has its own active ingredient namely;

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **NET TYPE** | **ACTIVE INGREDIENT** | **INTERNAL STANDARD** | **SOLVENT USED** | **ANALYTICAL INSTRUMENT** |
| MiraNet Combi | Alpha cypermethrin and Pipernoyl butoxide (PBO) | 4- Benzyl | Xylen | Gas Chromatography |
| Olyset Plus Net | Permethrin and Pipernoyl butoxide (PBO | 4- Benzyl | n- Heptane | Gas Chromatography |

#### Method development/ Validation

During my internship I was involved in the development analytical method for the detection of aflatoxin in corn flour. Although most of the instrument parameters were developed by Mr Festo Nicas, but I was also given a chance to work on the solubility of B-cyclodextrin in methanol, sodium hydroxide and acetonitrile all mixed together to make a series of the mixture solutions with different concentrations and pH so as to determine at what concentration and pH of these solutions will B-cyclodextrin dissolve best. To achieve this was necessary for the detection of the aflatoxin on the detector.

Another analytical method that I was involved was the determination of bendiocarb and Clothianidin dosage on filter paper treated with the bendiocarb and Sumishield solutions respectively, these were not newly developed analytical methods but were just the validation of the current analytical methods. Apart from basic analytical skills that I have improved during working on these analytical methods I was also given a chance to prepare a full working instruction and a Microsoft excel for the preparation, analysis and determination of the dosage of the bendiocarb in a filter paper treated with the bendiocarb formulation. The main knowledge learnt during this activity was the treatment of the filter paper of any size with the required dosage, extraction of the filter papers, analysis of the active ingredient by using HPLC and the calculation of the active ingredient content, dosage and percentage recovery by using Microsoft excel.

# **METHODOLOGY AND DISCUSSION**

Main analytical instrument learned during the whole period of internship include HPLC, GC and SPECTROPHOTOMETER.

## **High Performance Liquid Chromatography (HPLC)**

### Overview

High-performance liquid chromatography is an instrument in analytical Chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent (mobile phase) containing the sample mixture through a column filled with a solid adsorbent material (stationary phase). Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. There are two types of HPLCs depending on the type of column used; Normal phase HPLC and reverse phase HPLC. In normal phase HPLC the stationary phase consists of polar material such as silica and the mobile phase consists of a non-polar solvent while in reverse phase HPLC the stationary phase consists of a non-polar material while the mobile phase consists of a polar solvent. The mobile phase can be a mixture of different solvents mixed on specific ratios depending on the solubility of the analyte in the sample mixture in each of the solvent. Polarity of the mobile phase can be adjusted or left to remain constant during analysis depending on the method used for the analysis. When polarity of the mobile phase remains constant during separation it is called isocratic elution and when the polarity of the mobile phase is being changed during the separation it is called gradient elution. Different detectors can be used to detect the analyte after separation such as UV detector which is a universal detector since it detects a wide range of compounds, Fluorescence detector which specifically detects compounds that have fluorescence property.

### Operation and Mode of action.

After sample preparation and extraction, a small amount of sample solution is filtered by using membrane filter with a pore size of about 0.45 micrometer in diameter. The purpose of filtering the sample solution before analysis is to reduce the chance of column blockage by the particles in the sample solution that are of no interest, therefore the pore size of the membrane filter used at least has to be ten times less than the column particle size, for example a membrane filter of 0.45 micrometer in diameter can be used to filter a sample solution that passes through a column of 5 micrometer particle size. For the safety of the main column guard column can be used which also has the pore size even smaller than the main column so as to avoid blocking of the main column. Sample solution is filtered into auto-sampler vials and then the vials containing the filtered samples solution are arranged into the auto-sampler tray for further analysis. In many times it is likely to find bubbles in the channel of each solvent of the mobile phase. Presence of bubbles may cause the fluctuation of column pressure and hence reducing the efficiency of the separation process and hence it is crucial to remove all the bubbles in each of the channel. Bubbles are removed by the process called ‘Purging’. During purging the pump valve is rotated in the anti-clockwise direction so as to allow the mobile phase not to flow through the column but to pass straight into the waste tank. Now each channel is allowed to run separately at a composition of 100% until all the bubbles in the channel are removed by the degasser system. If the bubbles are still present after around 10 minutes of purging that channel the flow rate can be increased to enhance the flow. If the bubbles are still present then the membrane filter at the end of the channel can be changed and flow rate can be increased simultaneously. In most case the above techniques help to remove the bubbles from the channels.

In HPLC each sample to be analyzed has its own analytical method that has been developed specifically for the analysis of that sample. A method is a ….A suitable analytical method is developed only after evaluating the major and critical separation parameters of chromatography such as detection wavelength for the detector, stationary phase, column temperature, flow rate, mobile phase composition, elution mode, injection volume, analysis time etc. Before any analysis starts the analytical method for that particular analyte has to be launched in the HPLC software, when the method is launched the HPLC software will assume the parameters as per method instruction. From there a sequence for the analysis of the samples has to be created. A sequence is a set of information concerning the sample identity and location in auto-sampler tray given to the HPLC software. A sequence contains sample name, sample location in the auto-sampler tray and a number of injections per each auto-sampler vials. A new sequence can be created or an existing sequence can be edited to fit the information of the current samples. When creating a sequence is done the sequence has to be saved and if any changes were made on the method the new method parameters have to be saved also. The instrument can then left to run with the launched method for around 10 minutes for column stabilization. During these 10 minutes pressure of the column has to be observed if it is around the required range. An optimum pressure play a key role in the separation process, if the pressure is above normal then it is likely there are small particles stuck within the column of which they will affect the separation of our analyte of interest, also high pressure can result into dislocation of the packing material (stationary phase) in the column which will reduce the efficiency of the column and hence it is important to make sure that the pressure of the column can be tolerated before analysis starts. To remove the particles stacking within the column, the column can be reversed and the flow rate can be increased simultaneously so as to flush the particles out of the column. Another crucial aspect to look at before analysis is to check whether the present volumes of the solvents are enough for the whole analysis, because adding of the solvent before analysis finishes will affect the accuracy and precision of the result. To know this, the flow rate and the analysis time of all the samples together have to be taken into account. When pressure is optimum HPLC software can be commanded to run the sequence for the analysis.

### Quantitation and Interpretation of the Results

The quantitation of the samples after being analyzed by the HPLC depends on the peak areas of the samples displayed in the chromatogram.

Mathematically;

Peak Area ∝ Concentration

Peak Area = K x Concentration

The proportionality constant K is known as Response Factor (R.F)

R.F = Peak Area\Concentration

To obtain R.F we have to consider the concentration of the active ingredient, peak area of the active ingredient (A.I) and peak area of the internal standard (I.S) in the standard solution;

R.F = mass x purity x peak area of I.S/Volume x peak area of A.I

To obtain the concentration of the A.I in the sample solution;

Concentration = R.F x Peak Area of A.I x Volume of extraction

## **Gas Chromatography (GC)**

### Overview

Not far from HPLC, Gas chromatography is also an analytical instrument used to separate, identify, and quantify each component in a mixture. Unlike HPLC, gas chromatography uses a carrier inert gas instead of a mobile phase that caries the sample through the column to the detector. In GC the sample has to be volatile and stable at high temperature. Temperature acts as a key factor for the separation process. The stationary phase can be liquid or solid at its operating temperature and for this case a GC can be known as gas-solid chromatography or gas-liquid chromatography. A viscous polymer is coated as a thin film either directly on the inside wall of the tubing (wall-coated open tubural (WCOT) column) or onto a solid support material, which is packed tightly into the separation column (packed column). Separation occurs because sample components partition between the stationary phase and the mobile phase (carrier gas) according to the equilibrium expression called the distribution constant.

### Operation and Mode of action.

After switching on the hydrogen generator, nitrogen gas and the GC system itself the system is left to stabilize itself for around 10 minutes then slope test follows. A slope test is also known as a baseline analysis. It basically indicates where the area of the peak has to be integrated from. The higher value of slope test indicates that the column is not yet stable and it should be given sometime before analysis starts. A good slope test value has to be around 1000.

As explained above on HPLC part, also GC uses an analytical method and sequence to analyze samples and has the same way of interpretation and quantification of the results.

The table below shows the main difference between HPLC and GC

|  |  |  |
| --- | --- | --- |
|  | HPLC | GC |
| Sample | Volatile/non-volatile, low/high molecular weight, stable at room temperature, soluble in liquid phase. | Volatile, low molecular weight, stable at high temperature. |
| Column | Short and wide, packed column. | Long and narrow, packed column, capillary column |
| Mobile Phase | Solvent mixture, gradient, pressure required. | Pure inert gas, no gradient, no pressure. |
| Optional detectors | UV-Vis,FLD | FID,MS, ECD |

## **SPECTROPHOTOMETER**

This is an instrument that measures the intensity of light absorbed (absorbance) as it passes through a sample solution. With the spectrophotometer the amount of a known substance can be determined by measuring the intensity of light detected, it can also be used to measure at what wavelength a certain analyte has a maximum absorption of light. A mathematical relation between the intensity of the light absorbed and the concentration of the sample is given by the formula below;

Absorbance = є x concentration x thickness of the cuvet

Where;

Є is a constant known as absorptivity

The main components in this instruments are; a light source, optics to deliver and collect the light and a detector. Example of an experiment in which spectrophotometry is used is the determination of the equilibrium constant of the solution. A certain chemical reaction within a solution may occur in a forward and backward direction, where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of the reactants and products at this point, the light transmittance of the solution can be tested using spectrometry. The light of that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

## ISO 9001:2015 (QUALITY MANAGEMENT STANDARD)

This is a quality management standard when an organization needs to demonstrate its ability to consistently provide products and services that meet customer and applicable statutory and regulatory requirements. It aims to enhance customer satisfaction through an effective application of the system and regulatory requirements.

### Benefits of ISO 9001:2015

* It improves quality of services, products, efficiency and productivity of the company.
* Improves communication and awareness within and outside of the organization.
* Greater focus on continual improvement of the company.
* Enhances international recognition, employee confidence and gives a competitive edge to the company.
* It provides a good framework of the company and creates more satisfied customers.

### Implementation of ISO 9001:2015

The implementation of ISO 9001:2015 basically follows a system known as a P-D-C-A circle. The letters stand for Plan-Do-Check-Act. This is an iterative design and management method used in business for the control and continuous improvement of process and products. The PDCA circle is true to all elements and requirements of the quality management system. The principles of the PDCA circle can be maintained throughout all of the daily business aspects. It can be used formally and informally. The PDCA circle never ends, it is a continual improvement process. The circle is not one of the ISO 9001:2015 standard requirements but the standard definitely promotes the approach of the PDCA circle and it is an effective tool for achieving its requirements, especially the requirements from Clause 10.0; continual improvement.

### Clauses of ISO 9001:2015

ISO 9001:2015 has 10 clauses. Clause 1 to 3 describe the scope, normative references, terms and definitions of the ISO 9001:2015 itself.

#### CLAUSE 4.0: CONTEXT OF THE ORGAISATION

This clause consists for 4 sections;

1. Section 4.1: Understanding the organization and its context.
2. Section 4.2: Understanding the needs and expectations of interested parties.
3. Section 4.3: Determining the scope of the quality management system.
4. Section 4.4 : Quality management system and its processes

This clause explains about knowing

* The inputs required and outputs expected from the processes.
* The sequence and interaction of these processes.
* The criteria for measurements and related performance indicators to ensure an effective operation and control of the process.

#### CLAUSE 5.0: LEADERSHIP

This clause consists for 3 sections;

1. Section 5.1: Leadership and commitment
2. Section 5.2: Quality policy
3. Section 5.3: Organizational roles, responsibilities and authorities.

This clause explains about

* Leadership accountability.
* Establishment of quality objectives.
* Ensuring quality policy is communicated, understood and applied.
* Promoting awareness of the process approach and risk based thinking.
* Ensuring the resources needed for QMS are available.
* Ensuring the achievement of the QMS intended results.
* Promoting improvement.

#### CLAUSE 6.0: PLANING

This clause consists for 3 sections;

1. Section 6.1: Actions to address risks, operations and opportunities.
2. Section 6.2: Quality objectives and planning to achieve them,
3. Section 6.3: Planning of changes.

This clause explains about

* Consistency of the quality objectives with the quality policy.
* Identifying resources to achieve the quality objectives and a responsible person
* The planned quality objectives have to be measurable and evaluated.

#### CLAUSE 7.0: SUPPORT

This clause consists for 5 sections:

1. Section 7.1: Resources
2. Section 7.2: Competence
3. Section 7.3: Awareness
4. Section 7.4: Communication
5. Section 7.5: Documented information.

This clause explains about

* Resources; people, process environment, infrastructure and knowledge.
* Competence; knowing the competence level of the workers, the competence needed for achieving quality objectives
* Experience and any trainings needed.

#### CLAUSE 8: OPERATION

This clause consists for 7 sections:

1. Section 8.1: Operational planning and control
2. Section 8.2: Requirements for production and services.
3. Section 8.3: Design and develop products and services.
4. Section 8.4: Control of externally provided products and services.
5. Section 8.5: Production and service provision.
6. Section 8.6: Release of products and services.
7. Section 8.7: Control of non-conforming products and services.

This clause requires that an organization shall plan its operations, implement effective methods and control the processes so that the customer requirements are met and quality of products and services is delivered as expected by the customer. Operations can be optimized and effectively run when all the processes involved are planned, managed and controlled. Each of these processes is dependent on each other and interaction between each process may be very complex. Large organization like a manufacturing unit may have various departments handling operations such as sales and marketing, purchase of raw material, storage of raw materials, assembly line, quality control, storage and final delivery of the products to the customers.

#### CLAUSE 9.0: PERFORMANCE EVALUATION

This clause consists for 3 sections:

1. Section 9.1: Monitoring, measurement, analysis and evaluation.
2. Section 9.2: Internal audit.
3. Section 9.3: Management review.

The standard mandates determining what data needs to be collected and interpreted, and what results should be acted upon from a variety of inputs at various points in the quality management process. These results must later be verified (audited) and they must be subjected to the management’s direct review. This process also must itself be evaluated for effectiveness.

#### CLAUSE 10.0: IMPROVEMENT

This clause consists for 3 sections:

1. Section 10.1: General
2. Section 10.2: Non conformity and corrective actions
3. Section 10.3: Improvement.

This clause describes requirements for continual improvement in the quality management system by identifying nonconformities and taking corrective actions to recur the nonconformity by eliminating the root cause of the non- conformance.

It explains about;

* Determine and select opportunities for improvement.
* React to nonconformities and take action to eliminate the cause.
* Implement corrective actions as appropriate and review their effectiveness
* Keep records of nonconformities and corrective actions.
* Continually improve your QMS.
* Use the seven Quality Management principles to improve your ISO 9001 QMS.

# **CONCLUSION**

This internship has been an excellent and rewarding experience. I can conclude that there have been a lot I’ve learnt from my work at ATRC. Needless to say, the technical aspects of the work I’ve done have given me enough confidence and a solid foundation to start my career. As someone with no prior experience with complex lab instruments such a HPLC and GC whatsoever I believe my time spent on working with the technicians has given me enough knowledge that can help me stand out many lab scientists her in Tanzania. Two main things that I’ve learned the importance of are time-management skills and self-improvement.