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MINISTRY OF HEALTH

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

DIRECTORATE OF LABORATORY SERVICES

GUIDE ON SAMPLE SUBMISSION FOR ANALYSIS OF N-NITROSAMINES, DIETHYLENE GLYCOL AND ETHYLENE GLYCOL IMPURITIES IN PHARMACEUTICAL PRODUCTS

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Foreword

TMDA Quality Control Laboratory was established under section 14 (1) of Cap. 219 of the Tanzania Medicines and Medical Devices Act, 2003, to conduct quality control testing of medicines, medical devices and in-vitro diagnostic products.

The Laboratory is both WHO prequalified and ISO/IEC17025:2017 Accredited, which demonstrates the accuracy and reliability of the analytical results produced. This is supported by significant investments in modern equipment and staff knowledge. The various equipment makes it possible to test various parameters from a variety of samples including both medical and non-medical products. Therefore, our laboratories serve national and international customers including manufacturers, importers/exporters of all regulated products, researchers, regulatory bodies within Africa and the general public.

This guideline is prepared in response to requests from our customers to utilize the available equipment and expertise to test their products for various purposes. It should be noted that, TMDA being the national regulatory body will not test samples for the purpose of either batch/lot release or any regulatory submission for any manufacturer.

This guideline describes capability of the laboratory to test impurities specifically nitrosamines, diethylene glycol and ethylene glycol in medicines; raw materials and finished products. It also outlines the requirements that must be met when submitting samples for testing. Our customers are advised to read and make use of these services available at the TMDA Laboratory.

Dr. Adam M. Fimbo
Director General

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Dr. Danstan H. Shewiyo
Director of Laboratory Service

1.0 Introduction

The scope of samples analysed by the Tanzania Medicines and Medical Devices (TMDA) Quality Control Laboratory has been expanding over time due to various factors, including Government's efforts to enhance the quality of services provided by the laboratory, the need for quality pharmaceuticals for the public healthcare and the technological advancements worldwide. The demand for impurities testing has also increased due to emerging incidences of health catastrophes caused by presence of uchecked levels of impurities in finished pharmaceutical products and strict regulatory requirements to ensure the safety and efficacy of medicinal products.

In response to these, TMDA has developed this guideline to inform stakeholders about its capacity to test selected impurities, i.e., N-nitrosamines, diethylene glycol (DEG), and ethylene glycol (EG) in raw materials for manufacturing of pharmaceutical products and finished pharmaceutical products. These impurities have been selected due to recent research findings and reports in the media of their negative health effects. While research associated nitrosamines with cancer, DEG and EG have been reported to have caused death of 56 children in Gambia after using a contaminated cough syrup preparation.

The advancement of chemical knowledge along with the development of more sensitive and selective analytical techniques has consistently fuelled interest in assessing the purity of drugs by identifying impurities in both natural and synthetic products. This aligns with pharmaceutical industry standards, which have always emphasized the need for active pharmaceutical ingredients (APIs) to be of the highest purity.

As a result, the TMDA Quality Control Laboratory is now better equipped to serve the diverse needs of its national and international customers, including manufacturers, importers/exporters of regulated products, researchers, and regulatory bodies within Africa. Our commitment to continuous improvement and excellence in quality control ensures that we remain a trusted partner in safeguarding public health.

This guideline outlines methodologies and laboratory capacity on impurity analysis, emphasizing on the use of advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). These techniques provide high sensitivity and specificity which enable the precise identification and quantification.

The broad objectives of this guide therefore, is to ensure that pharmaceutical products manufactured in the country and imported ones conform to quality and safety standards for patient use.

2.0 Impurities

In the pharmaceutical industry, the presence of any component in raw material or drug product that is not a drug substance or excipient is an impurity and can significantly impact the quality, safety and efficacy of medications. Impurities are simply unwanted substances or chemicals that remain within the active pharmaceutical ingredients (APIs) or which develop during the formulation and storage of finished pharmaceutical products. Impurities are not intended to be part of the final product but they may arise from various sources throughout the manufacturing process.

The type, quantity, toxicity and potency of impurities found in drug substances or finished products products determines the ultimate safety of the finished pharmaceutical product. Therefore, the identification, quantitation and control of impurities are a critical part of the drug development process.

2.1 Types of Impurities in the Pharmaceuticals

2.1.1 Organic Impurities

These include starting material, by-products, intermediates, degradation products, and reagents used in the manufacturing process. For example, incomplete reactions during synthesis can leave residual starting materials, while side reactions can generate by-products. Also over time, the active ingredient or other components in the formulation may degrade, forming additional organic impurities. These impurities can

undesirably affect the safety and therapeutic effectiveness of the pharmaceutical product.

2.1.2 Inorganic Impurities

They arise from inorganic reagents, catalysts, heavy metals, or container materials used during the manufacturing process. They can also originate from residues of inorganic reagents or catalysts that were not completely removed after synthesis of APIs. Heavy metals, such as lead or mercury, might also be introduced from raw materials or equipment. These impurities can impact the stability and safety of the pharmaceutical product, necessitating applying control measures.

2.1.3 Residual Solvents

They are organic solvents used during the manufacturing process that are not completely removed from the final product. These solvents are used to dissolve, extract, or purify substances during synthesis. However, after use they must be removed because their presence can be harmful if ingested.

2.2 Sources of impurities in pharmaceutical products

Impurities in pharmaceutical products can originate from several sources throughout the manufacturing process. For example, raw materials which include starting materials and excipients, may introduce impurities if they contain contaminants. Also, during the synthesis of the active API, some chemical reactions produce by-products; the remains from residual solvents, reagents and catalysts in the final product can be the source of impurities. Furthermore; the API and excipients or container closure system can degrade over time or reacting forming additional impurities.

Impurities can also occur as a result of cross-contamination from other products due to inadequate cleaning of manufacturing equipment or from environmental factors such as dust and micro-organisms. They can be from packaging materials like plasticizers, adhesives, and rubber components which leak into the product, especially under improper storage conditions. Transportation as well may contribute to impurity formation due to variations in temperatures, humidity, and handling. Additionally, using recycled materials or containers without proper cleaning can introduce contaminants.

2.3 Impact of Impurities

The presence of impurities in pharmaceutical products can have several detrimental effects. Safety concerns arise if the impurities are toxic, carcinogenic or allergenic, posing potential health risks to patients. Impurities can also affect the efficacy of the drug, reducing its therapeutic effectiveness and leading to suboptimal treatment outcomes. Additionally, these impurities can compromise the stability of the pharmaceutical product, resulting in a shorter shelf life and reduced potency over time.

2.4 The importance of impurities analysis

2.4.1 Public Health and Safety

To safeguard Tanzanians' health, it is essential to make sure pharmaceutical items are free from dangerous contaminants. There are major health concerns associated with impurities. For example, N-nitrosamines cause renal failure, cancer, and even death. Even at modest exposure levels, the recognized carcinogen N-nitrosamines can raise the chance of developing cancer. Somolarly, if consumed, DEG and EG are extremely poisonous and can result in severe poisoning, metabolic acidosis, depression of the central nervous system, and renal failure. To avoid such serious health risks, these contaminants must be carefully analysed and controlled.

2.4.2 Regulatory Compliance

Adhering to international and national regulatory standards is essential for the pharmaceutical industry in Tanzania. Regulatory bodies, such as the TMDA adopted guidelines from WHO for acceptable impurity levels to ensure that pharmaceutical products are safe for consumption. Compliance with these regulations is necessary to maintain the quality of the products for both the local and international markets.

2.4.3 Quality Assurance

It is crucial for pharmaceutical products to be of high quality for effective healthcare and for safety. Therefore; analysing impurities ensures that the products meet specified quality and safety standards. This quality assurance is essential for the therapeutic efficacy of medicines, ensuring that patients receive treatments that are both safe and effective.

2.4.4 Economic Impact

The pharmaceutical industry plays a significant role in Tanzania's economy. Ensuring the quality and safety of pharmaceutical products helps prevent costly recalls and legal issues that could arise from contaminated medicines. This in turn supports the industry's sustainability and growth which will contribute positively to the national economy.

On the other hand, patient safety prevents government from spending more in prolonged treatment of their citizens and helps manpower availability for work which contibute to economy growth.

2.4.5 Export Market

Compliance to the international standards is a key fundamental requirement for local pharmaceutical companies to export their products, this is because of high competition from big international pharmaceutical companies. Many countries have imposed strict requirement for the products imported to their countries to safeguard their people. Therefore, impurity analysis contribute to ensure products conform to safety requirements and hence, easy access to international markets.

2.5 Potential Health Risks of Impurities

This document focus on nitrosamines, DEG and EG which recently have been of interest.

2.5.1 N-Nitrosamines

These impurities belong to a group of potent carcinogenic compounds. In pharmaceutical products, N-Nitrosamine can be formed as impurities through various chemical reactions during manufacturing, usually involving the reaction of secondary amines with nitrites under acidic conditions. They have been found in a variety of pharmaceuticals products leading to recalls..

2.5.2 Ethylene Glycol Diethylene glycol

They are toxic compounds when ingested. In industrial applications, they have several uses such as antifreeze and coolant formulations, due to its effective heat transfer properties. However, when these contaminates pharmaceutical products they pose significant health risks.

One of the primary health issue is metabolic acidosis, where the body's pH level becomes dangerously acidic, disrupting normal physiological functions. This condition results from the metabolism of these into toxic metabolites such as glycolic acid and oxalic acid. Additionally, central nervous system (CNS) depression is a major risk manifesting as symptoms ranging from dizziness and headache to severe cases of coma and respiratory failure. Furthermore, they are associated with severe renal failure, caused by the formation of calcium oxalate crystals in the kidneys, which can lead to life-threatening complications.

2.6 Analytical techniques for testing

One of the most important steps in impurity management is the development of analytical techniques and acceptability standards for contaminants in pharmaceutical raw materials and finished products. Isolation, identification, and quantification are all part of impurity testing.

2.6.1 Gas Chromatography-Mass Spectrometry (GC-MS)

It is a highly effective analytical technique widely used for detecting trace levels of impurities such as N-nitrosamines, DEG and EG. This method begins with gas chromatography, which separates the different compounds within a sample in the column. The separated compounds are then introduced into a mass spectrometer, which identifies and quantifies them based on their mass-to-charge ratios.

The high sensitivity of GC-MS allows for the detection of these impurities at very low concentrations. This is important for ensuring that pharmaceutical products meet safety and quality standards. In addition to that, the instrument has a high specificity which ensures that even trace quantities can accurately be identified without interference from other substances in the sample.

2.6.2 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

It combines the advanced separation abilities of liquid chromatography with the powerful detection capabilities of tandem mass spectrometry. This technique is especially effective in identifying and quantifying impurities in complex pharmaceutical matrices, such as those found in drug formulations that contain multiple components.

The process begins with liquid chromatography, which separates different compounds in a sample based on their chemical properties. Once separated, the compounds are introduced into the mass spectrometer, where they are detected and analysed based on their mass-to-charge ratios.

The MS/MS aspect involves multiple rounds of mass spectrometric analysis, allowing for the fragmentation of molecules and providing detailed structural information. This enhances the ability to accurately identify impurities, even those present in very low concentrations. The high sensitivity, precision and accuracy of LC-MS/MS ensures that trace levels of N-nitrosamines, DEG, and EG impurities, can be detected and quantified.

2.6.3 High-Performance Liquid Chromatography (HPLC)

It is a widely used analytical technique for separating, identifying, and quantifying various impurities in pharmaceutical products. The method involves injecting a liquid sample into a column containing adsorbent material. As the sample passes through the column, different compounds in the sample interact with the adsorbent material. Due to different affinity to the stationary and mobile phases, effective separation occurs based on their chemical properties.

2.6.4 Gas Chromatography with Flame Ionization Detection (GC-FID)

This is a widely used analytical technique for detecting and quantifying organic compounds. The method works by separating the different compounds in a sample through gas chromatography, which involves passing the sample through a column filled with an adsorbent material. As the sample passes through the column, its components are separated based on their boiling points and interactions with the adsorbent material or stationary phase.

Once the compounds are separated, they are directed into a hydrogen flame within the FID. In the FID, the organic compounds are burned, producing ions as a result of combustion. These ions generate a current, which is then detected and measured by the detector. The intensity of the current produced is proportional to the amount of each compound present in the sample, allowing for quantitative analysis.

2.6.5 Gas Chromatography (Capillary GC)

It employs narrow-bore capillary columns to achieve high-resolution separation. These columns are typically very thin, with an internal diameter ranging from 0.1 to 0.5 mm, which provides a large surface area for interaction between the stationary phase and the sample components. As the sample passes through the column, each compound interacts differently with the stationary phase, resulting in their separation based on their chemical properties and boiling points.

This technique is particularly useful for analysing organic compounds which are volatile. Capillary GC offers enhanced accuracy and reliability by providing sharp and well-defined peaks for each separated compound, making it easier to identify and quantify even trace levels of impurities.

3.0 TMDA Laboratory Capacity for Impurity Analysis

TMDA has three laboratories located in Dar es Salaam (Main Laborayroy), Mwanza (Lake Zone Laboratroy) and Dodoma (TMDA headquarter). These laboratories are equipped to perform comprehensive pharmaceutical analysis including analysis impurities in pharmaceutical products. The capacities and capabilities of the TMDA laboratories are as described below:

3.1 Advanced Analytical Instruments

The laboratory is equipped with advanced analytical instruments such as GC-MS, LC-MS/MS and HPLC. These instruments enable the laboratory to conduct different tests, ensuring that pharmaceutical products are safe, effective and of quality. Such utilization of advanced technologies, the laboratory is able detect minute levels of contaminants, which is essential for maintaining high quality standards products and protecting public health.

3.2 Skilled Personnel

The TMDA laboratory is staffed with highly trained analysts who are proficient in operating advanced analytical instruments and interpreting analytical data. These bring a wealth of knowledge and expertise to the laboratory, ensuring accurate and reliable results. The laboratory staff receive a continuous professional development and training which are integral to the laboratory's operations, ensuring that the team remains current with the latest analytical techniques. This commitment to ongoing education and skill enhancement allows the staff to effectively utilize the instruments and methodologies, maintaining the standards for high precision and accuracy in the analyses.

3.3 WHO- prequalification

Being a WHO-prequalified, means that the TMDA laboratory adheres to international standards and requirements and is competent in its functions. This prequalification signifies that the lab consistently operates with high levels of reliability and compliance to International standards. The laboratory also follows quality control measures and utilizes standard analytical techniques to ensure that the results produced are precise and accurate. This compliance with WHO standards boosts the credibility of the laboratory. Furthermore; WHO uses the TMDA laboratory as a reference laboratory for several pharmaceutical analyses.

3.4 Comprehensive Testing Capabilities

The laboratory can handle a wide range of pharmaceutical products, including raw materials, tablets, capsules, syrups, and other forms, both human and veterinary medicines. This extensive capability ensures that diverse pharmaceutical formulations are thoroughly analysed ensuring their safety, efficacy, and compliance with regulatory standards.

3.5 Research and Development

The TMDA laboratory engages in ongoing research and development activities to continually improve its analytical methods and stay up to date with current methods. Research ensure that the laboratory remains at the forefront of analytical science,

capable of detecting even the most elusive impurities. This proactive approach allows the lab to refine existing techniques and develop new methodologies, enhancing its capacity to ensure the safety and efficacy of pharmaceutical products. Collaboration with international research institutions and regulatory bodies further supports these efforts, fostering innovation and the adoption of best practices. Through continuous research and development, the TMDA laboratory upholds its commitment to high-quality analysis and public health protection.

3.6 Subscription to up-to-date Pharmaceutical Methods

Staying updated with the latest monographs is essential for the TMDA laboratory to accurately and reliably analyse impurities. By subscribing to current international pharmaceutical methods, the laboratory gains access to current analytical techniques and methodologies. This continuous access ensures that the laboratory can keep with the latest developments in analytical science, enhancing its ability in the analysis of impurities.

3.7 Submission of Samples for Analysis and Testing Costs

Domestic and internation stakeholders are invited to submit their samples for impurities testing, at the TMDA Quality Control Laboratories located in Dar es Salaam (main laboratory), Mwanza (Lake Zone Laboratory) amd Dodoma. Application for testing is done using the Test Request Form available at TMDA website (www.tmda.go.tz). Detailed information is available in the Guideline for Submission of Human and Veterinary Samples available online on the same website.

Testing costs applicable are stipulated in the current Fees and Charges Regulations, available at TMDA website at (www.tmda.go.tz).

4.0 References

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