QUALITY OF SELECTED MEDICINES IN ETHIOPIA:
ANALYTICAL AND REGULATORY CONTRIBUTIONS

Thesis submitted to obtain the degree of Doctor in Pharmaceutical Sciences

Sultan SULEMAN

Promoter
Prof. Dr. Bart DE SPIEGLEER
QUALITY OF SELECTED MEDICINES IN ETHIOPIA: ANALYTICAL AND REGULATORY CONTRIBUTIONS

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Master of Science in Pharmaceutical Analysis and Quality Assurance

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2016

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Ghent, 10 February 2016

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The author
Sultan Suleman
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Sultan Suleman
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<td>AAS</td>
<td>Atomic Absorption Spectroscopy</td>
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<tr>
<td>ACN</td>
<td>Acetonitrile</td>
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<td>ACT</td>
<td>Artemisinin-based Combination Therapy</td>
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<td>ADR</td>
<td>Adverse Drug Reaction</td>
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<td>AHA</td>
<td>9,10-anhydroartemisinin</td>
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<td>ALB</td>
<td>Albendazole</td>
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<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
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<td>ART</td>
<td>β-artemether</td>
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<td>As</td>
<td>Peak asymmetry</td>
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<td>ASTM</td>
<td>American Society for Testing and Materials</td>
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<td>ATMP</td>
<td>Advanced Therapy Medicinal Products</td>
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<td>ATP</td>
<td>Analytical Target Profile</td>
</tr>
<tr>
<td>AU</td>
<td>Absorbance Unit</td>
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<tr>
<td>BCS</td>
<td>Biopharmaceutical Classification System</td>
</tr>
<tr>
<td>BET</td>
<td>Bacterial Endotoxin Test</td>
</tr>
<tr>
<td>BP</td>
<td>British Pharmacopoeia</td>
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<tr>
<td>BSTFA</td>
<td>N,O–bis(trimethyl-silyl)trifluoro-acetamide</td>
</tr>
<tr>
<td>BZ</td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>CAP</td>
<td>College of American Pathologists</td>
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<td>CAPA</td>
<td>Corrective and Preventive Action</td>
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<td>CCF</td>
<td>Central Composite Face-centred</td>
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<td>CFU</td>
<td>Colony Forming Unit</td>
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<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
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<tr>
<td>CN</td>
<td>Criticality Number</td>
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<tr>
<td>Cps</td>
<td>Process capability index</td>
</tr>
<tr>
<td>CQA</td>
<td>Critical Quality Attribute</td>
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<tr>
<td>DACA</td>
<td>Drug Administration and Control Authority of Ethiopia</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode Array Detector</td>
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<tr>
<td>DB</td>
<td>Desbenzyl</td>
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<tr>
<td>DBK</td>
<td>Desbenzylketo</td>
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<tr>
<td>DHA</td>
<td>Dihydroartemisinin</td>
</tr>
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<td>DKA</td>
<td>Diketo aldehyde</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>DoE:</td>
<td>Design of Experiments</td>
</tr>
<tr>
<td>DruQuaR:</td>
<td>Drug Quality and Registration</td>
</tr>
<tr>
<td>EAC:</td>
<td>East African Community</td>
</tr>
<tr>
<td>EMA:</td>
<td>European Medicine Agency</td>
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<tr>
<td>ESI:</td>
<td>Electron Spray Ionization</td>
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<tr>
<td>EU/ml:</td>
<td>Endotoxin Unit per milliliter</td>
</tr>
<tr>
<td>EU:</td>
<td>European Union</td>
</tr>
<tr>
<td>FDA:</td>
<td>Food and Drug Adminstration</td>
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<tr>
<td>FDC:</td>
<td>Fixed Dose Combination</td>
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<tr>
<td>FID:</td>
<td>Flame Ionization Detector</td>
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<tr>
<td>FMEA:</td>
<td>Failure Mode Effect Analysis</td>
</tr>
<tr>
<td>FMHACA:</td>
<td>Food, Medicine and Healthcare Adminstration and Control Authority of Ethiopia</td>
</tr>
<tr>
<td>FPP:</td>
<td>Finished Pharmaceutical Product</td>
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<tr>
<td>GC:</td>
<td>Gas Chromatography</td>
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<td>GDP:</td>
<td>Good Distribution Practices</td>
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<td>GLP:</td>
<td>Good Laboratory Practices</td>
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<td>GMP:</td>
<td>Good Manufacturing Practices</td>
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<td>GPQCL:</td>
<td>Good Practices for Quality Control Laboratories</td>
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<td>GRP:</td>
<td>Good Regulatory Practices</td>
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<td>GTP:</td>
<td>Growth and Transformation Plan of Ethiopia</td>
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<td>HOA:</td>
<td>Horn of Africa</td>
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<td>HPLC:</td>
<td>High Pressure Liquid Chromatography</td>
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<tr>
<td>ICH:</td>
<td>International Conference on Harmonization</td>
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<tr>
<td>ICP-MS:</td>
<td>Inductively Coupled Plasma-Mass Spectroscopy</td>
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<td>IE:</td>
<td>Ion Exchange</td>
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<td>IEC:</td>
<td>International Electrotechnical Commission</td>
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<td>IGAD:</td>
<td>Intergovernmental Authority on Development</td>
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<td>IQ:</td>
<td>Installation Qualification</td>
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<tr>
<td>ISO:</td>
<td>International Organization for Standardization</td>
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<td>IU:</td>
<td>International Unit</td>
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<tr>
<td>JuLaDQ:</td>
<td>Jimma University Laboratory of Drug Quality</td>
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<tr>
<td>k':</td>
<td>Retention Factor</td>
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<tr>
<td>Lum:</td>
<td>Lumefantrine</td>
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<tr>
<td>lc:</td>
<td>Label Claim</td>
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<tr>
<td>LC:</td>
<td>Liquid Chromatography</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>LCL</td>
<td>Lower Control limit</td>
</tr>
<tr>
<td>LEI</td>
<td>Late Eluting Impurity</td>
</tr>
<tr>
<td>LMIC</td>
<td>Low and Middle Income Countries</td>
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<tr>
<td>LoD</td>
<td>Limit of Detection</td>
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<td>LoQ</td>
<td>Limit of Quantification</td>
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<tr>
<td>LQA</td>
<td>Laboratory Quality Attribute</td>
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<td>MCC</td>
<td>Medicine Control Council</td>
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<td>MDD</td>
<td>Maximum Daily Dose</td>
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<td>MEB</td>
<td>Mebendazole</td>
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<tr>
<td>MEDQUARG</td>
<td>Medicine Quality Assessment Reporting Guidelines</td>
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<tr>
<td>MOARD</td>
<td>Ministry of Agriculture and Rural Development</td>
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<td>MODR</td>
<td>Method Operable Design Region</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>MRA</td>
<td>Medicine Regulatory Authority</td>
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<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<tr>
<td>MΩ</td>
<td>Mega-Ohm</td>
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<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
</tr>
<tr>
<td>NDA</td>
<td>National Drug Authority</td>
</tr>
<tr>
<td>NO(A)EL</td>
<td>No Observed (Adverse) Effect Level</td>
</tr>
<tr>
<td>NTD</td>
<td>Neglected Tropical Diseases</td>
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<tr>
<td>OFAT</td>
<td>One-factor-at-a-time</td>
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<tr>
<td>PBD</td>
<td>Plackett-Burman Design</td>
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<tr>
<td>PDA</td>
<td>Photo Diode Array</td>
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<tr>
<td>PDCA</td>
<td>Plan, Do, Check and Act</td>
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<td>Ph. Eur.</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>Ph. Int.</td>
<td>International Pharmacopoeia</td>
</tr>
<tr>
<td>PQM</td>
<td>Promoting Quality of Medicines</td>
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<tr>
<td>QA</td>
<td>Quality Assurance</td>
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<tr>
<td>QbD</td>
<td>Quality-by-Design</td>
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<tr>
<td>QC</td>
<td>Quality Control</td>
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<td>QSAR</td>
<td>Quantitative Structure Activity Relationship</td>
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<td>QTPP</td>
<td>Quality Target Product Profile</td>
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<tr>
<td>RH</td>
<td>Relative Humidity</td>
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<td>RHB</td>
<td>Regional states Health Bureau</td>
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<tr>
<td>RO</td>
<td>Reverse Osmosis</td>
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</table>
LIST OF ABBREVIATIONS AND SYMBOLS

RPN: Risk Priority Number
RRB: Regional Regulatory Bodies
RRF: Relative Response Factor
RRT: Relative Retention Time
Rs: Resolution
RSD: Relative Standard Deviation
RT: Reporting Threshold
S/N: Signal-to-Noise ratio
SADC: South African Development Community
SD: Standard Deviation
SFFC: Spurious/falsely labeled/falsified/counterfeit
SOP: Standard Operating Procedures
SST: System Suitability Test
STH: Soil-transmitted helminths infections (helminthiasis)
TFDA: Tanzanian Food and Drug Administration
THF: Tetrahydrofuran
TLC: Thin Layer Chromatography
TLP: Target Laboratory Profile
TNZ: Tinidazole
TOC: Total Organic Carbon
tR: Retention Time
TTC: Threshold of Toxicological Concern
UCL: Upper Control Limit
UK: United Kingdom
UPLC: Ultra Pressure/Performance Liquid Chromatography
URS: User Requirement Specifications
US-IOM: United States Institute of Medicine
USP: United States Pharmacopoeia
USP-MC: USP Medicines Compendium (previously Salmous; stopped March 2015)
UV: Ultra-violet
V/V: Volume by Volume
WHO: World Health Organization
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“Quality is never an accident; it is always the result of intelligent effort”

John Ruskin

(1819 - 1900, English Philanthropist)
CHAPTER I

INTRODUCTION

1. QUALITY OF PHARMACEUTICAL DRUGS: CONCEPTS AND DEFINITIONS

Quality is a universal concept which needs to be defined in terms of specific characteristics, which vary from product to product [1]. For example, for pharmaceutical products, parameters such as medicinal effect, toxicity, physical and chemical characteristics, taste and shelf life may be important [2]. Definitions and concepts of pharmaceutical drug quality have been evolving over the years. ICH defines pharmaceutical drug quality as the fitness-for-purpose of a drug substance or drug product, including attributes as identity, strength and purity, without undesired side effects [3]. Short et al. redefined pharmaceutical drugs quality in terms of risk by linking product characteristics to clinical attributes, whereby the risk that final product characteristics impose on the safety and efficacy of treatment can be modeled [4]. Quality specifications could be defined as critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities [5].

The quality of pharmaceutical drugs is a global concern ensuring optimal treatment from medicines, and is currently receiving increased attention in an era of globalization and generic manufacturing [6-8]. Quality assurance and control play an essential role in the pharmaceutical manufacturing process, by ensuring that patients are provided with medications that are safe, effective, and produced at a high level of quality appropriate to their intended use [9, 10]. The manufacturing process should ensure product quality which requires consideration of the physical, chemical, and biologic characteristics of all of the drug substances (e.g., the solubility of the drug substance can affect the choice of dosage form) and pharmaceutical ingredients to be used in manufacturing the product [11].

The drug and pharmaceutical materials must be compatible with one another to produce a drug product that is stable, efficacious, attractive, easy to administer, and safe. The product should be manufactured with appropriate measures of quality control and packaged in containers that keep the product stable. The product should be labeled to promote correct use and be stored under conditions that contribute to maximum shelf life [3].

Since packaging preserves the stability and quality of medicinal products as well as protects them against all forms of spoilage and tampering, and since the information on the labels, packaging and
leaflets provide the basis for appropriate use, WHO recommends that all medicinal products need to be packaged in containers that conform to prescribed standards with careful labeling [12]. Despite recent advances in the manufacturing sector, quality issues remain a frequent occurrence, and can result in recalls, withdrawals, or harm to patients [10].

After a pharmaceutical product leaves the manufacturer’s premises, distributors, procurement agencies (purchasers), dispensers, and users are responsible for maintaining the quality of the product through proper storage, transport, distribution, dispensing, and use. National medicines regulatory authorities are responsible for ensuring that manufacturers comply with current GMP requirements and execute the key regulatory functions that are required to maintain product safety, efficacy and quality throughout the supply chain until the end user; which may present a challenge for countries with limited resources [13].

There is growing concern that much of the developing world’s supply of medicines, in particular, its supply of anti-infective drugs is of poor quality, resulting in avoidable morbidity, mortality and drug resistance [14, 15]. There are different national and international efforts to counter poor quality medicines [15-19]. However, efforts to improve the quality of medicines in developing countries are being hampered by confusion over the terms used to describe different types of poor quality medicines [20]. There are two main categories of poor quality medicines: substandard and counterfeit. According to world health organization (WHO), substandard medicines are genuine medicines which have not passed the standards and quality testing protocols set for them. These standards and quality tests have been defined in the official pharmacopoeias such as the International, the European and the United States Pharmacopoeias [21]. A counterfeit or falsified medicine is one which is deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients, wrong ingredients, without active ingredients, with insufficient quantity of active ingredient or with fake packaging [21, 22].

It needs to be mentioned that the relationship between combatting counterfeit medicines, addressing safety, quality and efficacy issues, and enforcing privately owned intellectual property (IP) rights has become controversial. This debate between health and legal arguments lead to the concern that a wider definition of ‘counterfeit’ threatens the trade in generic medicines of assured quality on which many developing countries depend [23].

In the past, WHO distinguished between “substandard” and “counterfeit” medicines as defined above, but in March 2011, its member states chose to lump them together in the new term “substandard/spurious/falsely-labeled/falsified/counterfeit medical products” (SSFFC) [24]. However, placing all illegitimate medicines under the SSFFC umbrella gives the misleading impression that they are all deficient in the same way, when actually there are many possible deficiencies, each requiring
different solutions. Thus, Attaran et al. proposed new definitions, which refine the views of WHO member states as presented in Figure 1 [22].

An important strength of the new definitions proposed by Attaran et al. is that they do not draw on any IP questions or use the confusing word counterfeit. According to international intellectual property law, a “counterfeit” medicine is one that infringes a registered trademark by bearing an identical or near identical mark [25]. Deliberate counterfeiting is a private economic wrong, which can occur separately from or together with the public health wrong of poor medicine quality. Thus, it is a mistake (although a common one) to use the adjective “counterfeit” to refer to medicines that endanger public health [22]. “Substandard” medicines are those that for unintentional reasons do not meet the legally required quality specifications of a country’s regulators (usually a specialised medicine regulatory authority). As noted elsewhere, substandard medicines have various deficiencies and causes; for example, the raw ingredients may be of poor quality, errors may occur in manufacturing, or mishandling may cause the medicine to degrade unexpectedly rapid [26]. Each of these technical problems requires a different solution.

![Figure 1](image)

**Figure 1.** A two-dimensional description of medicine quality and registration. (Attaran et al. 2012 BMJ 345:e7381)

As presented in Figure 1, unregistered medicines may be of good quality. Accordingly, good quality unregistered medicines are separated from other types of illegitimate drugs. Hence in spite of the strengths, the new definitions of poor quality medicines proposed by Attaran et al. have some drawbacks: (1) it does not have clear definitions and classifications for unregistered medicines of poor quality; (2) it catagorises the unregistered drugs of good quality as illegitimate only due to
18

diversion or theft, where these medicines could in fact fulfil the quality standards but diversion and theft have no guarantee for good transport and/or storage; (3) those unregistered medicines of good quality can also be called falsified as regard to their history since they are not following the legal/formal supply anymore or can be re-introduced wrongfully; and (4) in the worst case scenario, those unregistered medicines can be tampered with and can be classified as falsified as regards to their identity and/or source.

“Unregistered” medicines are those not legally approved by the local drug regulatory authority to be imported or sold in the country [27]. Unregistered medicines are vulnerable to quality failures and are more prone to substandard quality as well. They do not enter the market through reputable channels and are often transported under poor conditions; situations where quality problems can easily go undetected [28]. With unregistered medicines (which are also often substandard), some aspect of the medicine does not meet the regulator’s legal requirements. Generally, such products also deviate from the regulator’s approved packaging/labelling (correct and legible labeling of active ingredients and strength, expiration date, batch number, manufacturer, and country of origin) [22]. Since the definition of counterfeit medicines affects IP [25], the term “falsified” medicines is used to distinguish the issue from IP violations, thereby protecting and facilitating the trade in affordable generics. Thus, a falsified medicine is defined to be one that falsely represents the product’s identity or source or both. Thus, a falsified medicine may or may not contain the wrong ingredients, ingredients of low quality or in the wrong doses, but they are deliberately and fraudulently mislabelled with respect to their identity or source, and thus have fake packaging [29].

Similar to substandard medicines, the falsified medicines are unlawful in violating the regulator’s quality specifications, but what truly defines and distinguishes them is criminal intent. Thus it takes more than a negligent breach of the regulator’s legal requirements to make a medicine falsified; there must also be a deliberate intent to deceive [22].

Therefore, public health-oriented concepts and definitions (avoiding unnecessary controversy and thus better enabling governments, companies, advocates, and the health professions to protect public health) must be used in consensus to improve the overall quality of the world’s supply of medicines. Moreover, in order to avoid such confusions, the semantics ‘poor quality’ is recommended to represent substandard and/or counterfeit medicines [19, 22].

2. THE IMPACT OF POOR QUALITY PHARMACEUTICALS

Access to medicines of good quality is a basic human right of everyone: it improves the chances of successful treatment for the individual patients and promotes better outcomes for public health in
general [30-32]. The presence of poor quality medicines in the market is a global threat on public health, especially in developing countries by critically challenging the efforts of treatment and control of diseases [33, 34].

Medicine quality problems could be fatal in extreme clinical outcomes and have also been associated with severe economic consequences. More than 700,000 deaths from tuberculosis and malaria have been strongly linked with ineffective poor quality medicines worldwide [35, 36]. Mortality has also been reported after glycerol contamination in different countries, heparin contamination in the United States and Germany, and after using sexual enhancement drugs adulterated with large contents of hypoglycemic drugs in Singapore [37-41].

The WHO estimates that around 10% of all global pharmaceutical supply is counterfeit and substandard, reaching up to 50% of the supply in developing countries and as low as 1% in the developed world. About 100,000 deaths a year in Africa are linked to the counterfeit drug trade [35, 42-44].

The impressive globalization of the pharmaceutical market that characterized the last two decades has led to an increased risk of poor quality medicines, where the quality of medicines largely depends on the countries’ level of regulation [45]. Counterfeit drugs are estimated to be less than 1% of the market in strictly regulated countries, but have been reported ten times more often within the last 5 years in the United States [46, 47]. In Europe, the number of counterfeit products increased by 118% and their confiscations had a relative increase by 57% within one year (2007-2008) [47], a trend confirmed by more recent estimations. In developing countries, counterfeit drugs are estimated to account for between 10% and 30% of all drugs sold, with rates higher than 30% in some regions of South-east Asia, Sub-Saharan Africa and Latin America [48]. In a recent literature review 44%, 30% and 9% of 163 counterfeit antibiotics were detected in South-East Asia, Sub-Saharan Africa and Europe/North America, respectively [47]. Overall, up to 60% of antimicrobials in Africa and Asia may have low quality [48].

The 2015 Nobel Prize in Physiology or Medicine awards discoveries regarding novel therapies for some of the most devastating parasitic diseases. Youyou Tu, a Chinese professor born in 1930 in China, was awarded for her discovery of artemisinin, a drug that has significantly reduced the mortality rates for patients suffering from malaria. However, the circulation of poor quality antiparasite medicines including artemisinin containing combination therapies (ACT) is pouring cold water on similar innovative endeavors and public intervention strategies. Studies indicated 48% mebendazole [49] and 37% antimalarial medicines of poor quality in Nigeria [50]. More than 12% poor quality antimalarial medicines were reported in Tanzania [51], while very poor quality anthelmintic preparations were reported in Kenya [52]. These findings reveal the importance of
addressing similar problems with respect to selected antiparasitic medicines of public health importance in Ethiopia.

Despite different efforts of countering the problems of poor quality medicines, recent literatures still report the widespread presence of poor-quality medicines [53]. They also show that there is an urgent need to develop more accurate and standardized methods for mapping their distribution and characteristics. The knowledge generated by well-designed surveys will inform national regulators for identifying and correcting the vulnerabilities of the pharmaceutical supply chain [54].

To counter the problems associated with poor quality medicines, correct determination of the prevalence is of paramount importance [15]. However, determination of the epidemiology of poor quality medicines is challenging either due to inaccurate sampling methodologies or randomization problems. Moreover, the tests and analytical techniques were often not adequate or insufficient for a complete quality assessment, e.g. the important but relatively expensive dissolution test is not included in most studies [15, 53]. Therefore, implementation of cost-effective, simple and accurate analytical methods that can identify the distribution of poor quality medicines together with effective regulatory mechanisms is of paramount importance to reduce this poor-quality problem.

3. COUNTERING POOR QUALITY MEDICINES: ANALYTICAL AND REGULATORY CONTRIBUTIONS

As a serious but generally neglected public health problem, poor quality medicines are urged to be fought [55]. Various intervention approaches are used in combatting poor quality medicines including effective legislation and regulatory mechanism to establish a robust quality assurance system that ensures quality of medicines throughout the pharmaceutical supply chain [56, 57]. To measure is to know, and hence, there is a need for fast, efficient, simple, robust and transferable analytical methods that can be used for detection and analysis of poor quality medicines [58]. Moreover, independent medicine quality control laboratories for continuously monitoring the quality of medicines throughout the supply chain, i.e. from manufacturing to consumer use, are desperately needed [59]. El-Jardali et al. developed a conceptual framework for all the intervention strategies against poor quality medicines, presented in Figure 2 [60].
Figure 2. A framework for the different strategies against poor quality medicines. Note: The shaded cells in the framework portray areas where evidence about the intervention exists. (El-Jardali et al. 2015 BMJ Open 5:e006290).

Factors contributing to poor quality medicines include lack of legislation and weak or absent regulatory authorities, and lack of quality control laboratories or field tests.

**Analytical contributions**

Combatting poor quality medicines requires quality investigation of medicines, and this is based on the availability of robust and reliable analytical methods, which preferably should be as fast and cheap as possible while possessing high information content [61]. There are diverse techniques to analyse pharmaceuticals and identify poor quality medicines, going from simple visual inspections, testing of physical properties up to chemical evaluations involving chromatographic separation techniques with different detection modes [58, 62].

A three-level approach proposed by Pribluda et al. consists of different quality control procedures to detect poor quality medicines. Level 1 includes inspection to determine the quality of packaging and labelling. Level 2 encompasses methods that can be done in the field. Level 3 requires the equipment of an established laboratory to determine drug quality according to established specifications [59].

The visual inspections (Level 1) are a quick and easy way to detect poor-quality medicines. They are focussed on labeling, packaging integrity, package information and the appearance of the medicine. This visual inspection can make use of comparison of the sample to the authentic product if available to verify its falsified status. It is the standard first step in any medicine quality analysis [63].

Various screening tests could be used to perform Level 2 analyses. Several methodologies are currently available for this purpose, such as Raman spectroscopy, infrared spectroscopy, and Thin-Layer Chromatography (TLC). Taking into account price, availability of required supplies and extent of
information that can be gathered, promoting quality of medicines (PQM) currently recommends for this level the use of TLC and for solid dosage forms disintegration [59, 64, 65].

The Global Pharma Health Fund Minilab is a portable drug quality analysis toolkit consisting of equipment and instructions for TLC, chemical colorimetry, and disintegration tests, as well as a visual inspection protocol. Testing and inspection protocols and materials are included for more than 50 WHO essential medicines, including reference standards for 63 drug compounds [66, 67]. However, field tests are no substitute for definitive laboratory techniques; they cannot test all aspects of a product’s quality, including its drug content, impurity profile and dissolution profile.

Level 3 analyses use the most advanced tests and are the only ones that can assess compliance with the formal quality attributes and specifications established during development and approved by the competent authorities. These analyses are capable of evaluating content and impurities with more accuracy and precision and hence discriminating power, than those described above for level 2 testing [59].

The most common L3 analyses that apply to practically all dosage forms are tests for identification, strength/content (assay), and impurities (organic, inorganic, and/or residual solvents) and thus involve the use of high-tech instruments like high pressure/performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectroscopic techniques. HPLC represents the gold standard for chemical separation, quantification and identification [63]; and mass spectrometry (MS) has become an important tool for elucidating the structures of low level unknown impurities and pharmaceutical degradants because of its unique analytical features [68].

To reduce the costs, simplicity and speed of analysis is becoming increasingly important in the HPLC applications in routine quality assurance (QA)/quality control (QC) activities. High speed analyses without loss in separating power can be achieved by reduction of particle size of the column, typical characteristics of fused-core Halo® columns [69] stressing the importance of analytical techniques involving this fused-core technology in resource-limited settings.

Impurities present in the active pharmaceutical ingredient (API) have to be identified to make sure no mutagenic or toxic substances will be administered to patients. Drug product degradation profiles need to be established to guide stable formulation and provide suitable drug shelf life assessment. Drug regulatory agencies also have requirements for characterization of the impurity profile of a pharmaceutical. The analysis of these low level unknown impurities and degradants can be very challenging [70]; and thus the first step in a general analytical strategy is to measure the molecular weight (MW) of the unknown by suitable ionization methods, commonly carried out either by electrospray ionization (ESI) [71, 72].

Moreover, Baert and De Spiegeleer in their investigation of internet-pharmaceuticals recommended a cost-efficient tiered system (Figure 3), which starts with the simplest and inexpensive methods.
They proposed the tiered approach in assessing the different quality attributes related to the overall quality of internet obtained pharmaceuticals. In a first step, the product classification is compared with the regional and/or national legislation. Secondly, the labelling and packaging should be carefully examined for legal conformity. If more detailed chemical information is required, determination of the physicochemical properties of the pharmaceutical product should be a starting point, followed by non-destructive chemical testing. In the last stage, destructive analytical techniques, often in a remote laboratory, will be applied. Whenever non-conformity is found, the risk for public health is to be evaluated, e.g. by toxicity testing. Evidently, pharmaceutical products that do not comply should be withdrawn from the market and their origin traced back for legal action [73].

Apart from methods used in countering poor quality medicines and because some advanced techniques need a laboratory setting, WHO recommends access to pharmaceutical quality control (QC) laboratories which are committed to provision of quality analytical services of international standards [74]. With the vision of good quality medicines for every one, WHO launched medicines prequalification program in March 2001 starting with essential medicines and expanded to include pharmaceutical QC laboratories in April 2004 [75]. From the 39 total list of of prequalified QC laboratories published by WHO-Prequalification programme in 2015, only 8 are from WHO Africa region and no QC laboratory is prequalified from the horn of Africa [76] outlining the importance of such prequalified QC-laboratories to support the quality survey of essential medicines in the region.

In one way or the other, the analytical technique applied in routine QA/QC activities should be robust. Currently, robustness of analytical methods is easily evaluated by using a quality-by-design (QbD) principle [77]. As defined by Janet Woodcock in 2004, "QbD means that product and process performance characteristics are scientifically designed to meet specific objectives, not merely empirically derived from performance of test batches" [78]. International conference on harmonization (ICH) Q8(R2) defines quality-by-design (QbD) as a systematic approach to design and develop a product and/or service based on sound science and quality risk management [3]. It is already frequently applied in the pharmaceutical industry mainly in the development of pharmaceutical products [78-80] and analytical methods [81-85], but is not well established in the set-up of facilities like quality control (QC) laboratory triggering the importance of such scientific principles in such facilities.
Figure 3. Tiered flow chart for quality evaluation of internet pharmaceuticals. NC non-conformity. (Baert B and De Spiegeleer B 2010 Anal Bioanal Chem 398: 125-136).

Regulatory contributions

To protect public health, governments need to approve comprehensive laws and to establish effective national competent authorities to ensure that the manufacture, trade and use of medicines are appropriately regulated to ensure the quality, safety and efficacy of medicines used by appropriate patients [86]. Medicines regulation, operating within a legal framework, demands the application of sound medical, scientific and technical knowledge and skills. It also involves interactions between the various stakeholders (manufacturers, traders, patients, health professionals, researchers and governments). Key to an effective medicines regulation is the government’s political commitment including legal powers, human and financial resources, and appropriate strategies and methods to implement the various regulatory tasks [87]. A sound medicines regulatory system is essential to combat the problem of poor quality medicines [88]. However, according to WHO estimate, nearly a third of its member states currently have very limited
medicines regulation [89]. Assessment of medicines regulatory systems in sub-saharan Africa indicated that the overall regulatory system presented some critical weaknesses (Figure 4) [90], with QC-unassignment the largest relative weakness, and thus need to be strengthened.

Strengthening the medicine regulatory authorities (MRAs) including enforcement possibilities, improving quality of production, and facilitating the availability of relatively inexpensive medicines is likely to be key factors in improving the overall quality of medicines [14]. Support for MRAs in developing countries, and the development of regional pharmaceutical QC laboratories to allow the regulation of medicines supply is crucial to allow effective intervention on the problem of poor quality medicines.

Governments in low- and middle-income countries should support their regulatory agencies to develop strategic plans for compliance with international manufacturing and quality control standards. In the least developed countries, international organizations should support their efforts [91]. As poor quality medicines are a global problem and the capacities of national MRAs in developing countries is currently limited, they can only be combatted by regional and international collaboration. Attaran et al. [22] and Binagwaho et al. [88] thus proposed the need of a global treaty to guarantee sustainable progress towards higher quality medicines by bringing regulatory, technical, legal, and financial mechanisms together (Figure 5).

A binding international law under the form of a treaty on drug quality is needed to complement the existing trade treaties, putting public health on an equal plane. A successful treaty is considered a first step to counter the problem of poor quality medicines. The growing political energy to fight poor
quality medicines could, if wisely channelled into a treaty, provide new financing and reinforced capabilities against poor quality medicines [22].

![Figure 5](image-url). Elements of a treaty to improve the safety of medicines. The figure shows the linkages between treaty elements (green), directly supported actions (red), and supporting information exchanges (orange). (Attaran et al., 2012 BMJ 345: e7381).

Next to a treaty, regional co-ordination of medicines regulatory activities is another approach to overcoming resource constraints. Regional co-operation can help participating countries to share expertise and experience, support each other in implementing national drug strategies, and, ultimately, avoid duplication, thereby making the best use of scarce regulatory resources, very similar to the European regional regulatory systems. In Africa, the East African Community (EAC) and Southern African Development Community (SADC) are pursuing harmonization, supported by a new initiative called the African Medicines Registration Harmonization Initiative (AMRHI). The goal of AMRHI is to foster regionalization of certain aspects of medicines regulation within economic groupings in Africa [26, 92]. Such regulatory harmonizations and collaborative initiatives are however only deemed to be successful if the current national legal-regulatory status is well-known, internationally compared as well as to benchmark it versus internationally accepted standards.
4. OBJECTIVES OF THE THESIS

High quality medicines should be accessible to the community in developing countries to control diseases of public health problem. The spread of poor quality medicines worldwide and particularly in developing countries are seriously affecting the success of patient treatments. WHO reported that 6% of medicines worldwide are poor quality [93]. According to the US Food and Drug Administration (FDA), poor quality medicines may account for more than 10% worldwide; and up to 25% of all medicines in developing countries have low quality [94]. Therefore, to guarantee an access of populations to safe and sure medicines, it is important to set up appropriate measures that will allow evaluating and preserving the quality of those medicines. Different intervention approaches are used in combating poor quality medicines including effective legislation and regulatory mechanism to establish a robust medicines quality assurance system throughout the pharmaceutical supply chain [56, 57]; fast, efficient, simple and transferable analytical method that can be used for detection and analysis of poor quality medicines [58]; and medicine quality control laboratory for continuous monitoring of the quality of medicines [59]. Therefore, the global objective of this thesis is to increase the quality of medicines in Ethiopia.

In order to address this goal, different questions are put forward as objectives:

1. **Is it possible to set up a well functioning and an accredited pharmaceutical quality control laboratory in resource-limited setting?**
   A quality-by-design (QbD) principle will be applied to establish an accredited pharmaceutical QC laboratory in Ethiopia. Analogous to process/product QbD and analytical QbD, laboratory QbD will be introduced. Quality scheme based on WHO-prequalification supported by ISO/IEC 17025 2005 requirements will be applied for prequalification and accreditation. The **first objective** was thus to set up a well functioning and an accredited pharmaceutical QC laboratory in Jimma, Ethiopia.

2. **Could suitable analytical methods be developed for artemisinin-based anti-malarial medicines?**
   Artemisinin-based (ACT) anti-malarial medicines are recommended by WHO as a first-line treatment for malaria. However, the current artemisinin FDC products, such as β-artemether and lumefantrine, are inherently unstable and require controlled distribution and storage conditions, which are not always available in resource-limited settings. Moreover, quality control is hampered by lack of suitable analytical methods. Thus, there is a need for a rapid and simple, but stability-indicating method for the simultaneous assay of β-artemether and lumefantrine FDC products. The **second objective** was thus to develop suitable analytical methods for artemisinin-based antimalarial medicines.
3. **How is the quality of ACT anti-malarial medicines in Ethiopia?**

Ethiopia is one of the malaria-endemic countries in sub-Saharan Africa, where fixed-dose combination products of artemether and lumefantrine have been used for the treatment of uncomplicated Plasmodium falciparum malaria. However, there is a little existing knowledge about quality of ACT anti-malarial medicines currently available in the market. The third **objective** was therefore to verify the dichotomous quality of artemisinin-based fixed-dose combination anti-malarial medicines circulating in Ethiopia.

4. **What is the prevalence of poor quality medicines used for the treatment of neglected tropical diseases in Ethiopia?**

Access to medicines of good quality improves the chances of successful treatment for individual patients and promotes better outcomes for public health in general. However, the presence of poor quality medicines in the market is a global threat on public health, especially in developing countries by critically risking efforts of treatment and control of diseases in general and the NTDs in particular. Conventionally, medicine quality has been ignored in NTDs, though scattered reports show that serious problems exist. Therefore, the fourth **objective** was to determine the prevalence of poor quality medicines for soil-transmitted helminths and giardia in Ethiopia.

5. **How is pharmaceutical regulatory framework in Ethiopia?**

Effective and enforceable national regulations describing the manufacture and (re)packaging, export and import, distribution and storage, supply and sale, information and pharmacovigilance of medicines are required to consistently ensure optimal patient benefit. Expansion of pharmaceutical industries in many countries with advancement in transport technologies facilitated not only trade of genuine pharmaceutical products, but also the circulation of poor quality medicines across the globe. One of the different intervention approaches used in combating poor quality medicines is having effective legislation and regulatory mechanism. The fifth **objective** is thus to investigate the current regulatory frame of medicines in Ethiopia, looking at the challenges for future improvements aiming at strengthening the system which will increase the quality of medicines.

5. **THESIS OUTLINE**

The thesis outlines quality of selected medicines in Ethiopia by broadly exploring the evidence from both analytical and regulatory perspectives.
In Chapter II, the application of a quality-by-design (QbD) principle in setting up a functional and accredited pharmaceutical quality control laboratory is presented. The different concepts of QbD are presented; analogous to process/product QbD, a new concept of laboratory QbD is introduced.

In Chapter III, quality analytics of lumefantrine is presented. HPLC/UV/MS impurity profiling and in-silico toxicity evaluation results of the different lumefantrine impurities are addressed. Moreover, the development and validation of a new stability-indicating gas chromatographic (GC-FID) method is documented for the routine regulatory QC application in resource-limited settings.

In Chapter IV, the development and validation of a rapid, simple and suitable stability indicating HPLC-UV for the simultaneous determination of artemether and lumefantrine in fixed dose combination products is clearly presented. Moreover, the result of the dichotomous quality evaluation of artemisinin-based anti-malarial medicines circulating in Ethiopia is presented.

In Chapter V, nationwide quality survey results for two commonly used anthelminthic drugs (MEB and ALB) and one antiprotozoal drug (TNZ) in Ethiopia is presented. The analytical results are converted into conclusions using two systems: the traditional dichotomous pharmacopoeial specification-compliance based approach and the risk-based Taguchi quantitative desirability approach. Moreover, the prevalence of poor quality of the three medicines is clearly determined and presented.

In Chapter VI, the pharmaceutical regulatory approval system of Ethiopia is presented recommending risk-based regulatory mechanisms. It is clearly described that from legislative point of view, the medicines regulation system in Ethiopia fulfils the minimum criteria for effective medicines regulation. However, the high prevalence of poor quality medicines documented in chapter V reveal that the mere existence legislation and regulatory mechanism is not a guarantee unless strengthened for effective implementation of all the regulatory functions.

In summary, a graphical representation of the different aspects investigated in this work is given in Figure 6.
6. REFERENCES


[30] United Nations Human Rights Council: Access to medicines in the context of the right of everyone to the enjoyment of the highest attainable standard of physical and mental health; 23rd session, Agenda item 3, Promotion and protection of all human rights, civil, political, economic, social and cultural rights, including the right to development. 2013.


CHAPTER II

QUALITY-BY-DESIGN PRINCIPLES APPLIED TO THE ESTABLISHMENT OF A PHARMACEUTICAL QUALITY CONTROL LABORATORY IN A RESOURCE-LIMITED SETTING: THE LAB-WATER

"Quality in a service or product is not what you put into it. It is what the client or customer gets out of it."

Peter Drucker
(*1909 - †2005, Austrian-born American management writer)

Parts of this chapter were published:

ABSTRACT

Quality-by-Design (QbD) is defined as a systematic approach to design and develop a product/service based on sound science and quality risk management. It is already frequently applied in the pharmaceutical industry mainly in the development of pharmaceutical products as well as analytical methods, but is not well established in the set-up of facilities like quality control (QC) laboratory. Therefore, this research reports the application of some risk-based scientific QbD principles in the establishment of a pharmaceutical QC laboratory in a resource-limited setting with focus on the laboratory water.
CHAPTER II

QUALITY-BY-DESIGN PRINCIPLES APPLIED TO THE ESTABLISHMENT OF A PHARMACEUTICAL QUALITY CONTROL LABORATORY IN A RESOURCE-LIMITED SETTING: THE LAB-WATER

Main focus in this chapter:
- To establish a pharmaceutical quality control (QC) laboratory in Jimma University, Jimma, Ethiopia that can contribute to QC analytics of medicines to the Horn of Africa.
- To present a quality-by-design (QbD) based establishment for obtaining laboratory water.

1. INTRODUCTION

The term quality-by-design (QbD) was created in 1970s by the quality expert Joseph M Juran and popularized in the 1990s [1]. Within the pharmaceutical field, International Conference on Harmonization (ICH) Q8(R2) defines QbD as a systematic approach to development that begins with pre-defined objectives and emphasizes product and process understanding and process control based on sound science and quality risk management [2]. US Food and Drug Administration (FDA) describes QbD as one arm of the quality system based on building quality in the development phase and throughout a product’s life cycle [3]. QbD as applied in the pharmaceutical industry is thus defined as a risk-based and proactive approach to pharmaceutical development [4]. In general, these definitions indicate that quality must be designed and built into product or service [5-8].
The principles for the successful implementation of QbD for product development involve: identification of the product attributes, such as a quality target product profile (QTPP) and critical quality attributes (CQAs); design space (the relationship between process inputs and CQAs); a robust control strategy to ensure consistent process performance; and finally, ongoing monitoring to ensure robust process performance over the life cycle of the product [5, 6]. ICH Q8 (R2) defines QTPP as a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product. QTPP forms the basis of design for product development. Once QTPP has been identified, the next step is to identify the relevant CQAs. A CQA is defined as a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality [2].

The QbD principle applied to product development is called product QbD (pQbD). These same QbD principles have also been applied to the development of analytical methods [9-12]. The concept of QbD applied to analytical method development is known as analytical QbD (aQbD) [13]. Equivalent to pQbD, aQbD plays a key role in the pharmaceutical industry for ensuring the product quality. Analytical QbD has different tools such as analytical target profile (ATP) establishment, CQAs, risk assessment, method optimization and development with design of experiment (DoE), method operable design region (MODR) and control strategy [14]. It helps in the development of a robust and fit-for-purpose analytical method [15].

The ATP and MODR parallel the QTPP and design space defined for a product and its manufacturing process. MODR is a multidimensional space based on the method factors and settings that provide suitable method performance [15-20]. DoE incorporates a set of characteristics which are essential in aQbD and is used for screening of factors, process characterization and optimization of multiple responses [21, 22]. Therefore, the steps, tools, and approaches developed for application of QbD to manufacturing processes have analogous application in the analytical environment [23].

Applying the principles and concepts of pQbD and aQbD, a risk-based and robust quality management system can be built into quality control (QC) laboratories starting from establishment to provide enhanced flexibility and continuous improvement [18, 24] by reducing variations and producing consistent results.

QC laboratories should generate reliable and traceable analytical quality data that meet user requirement specifications (URS). To ensure this, the laboratory needs a well-founded, effective, comprehensive and defensible quality system in place [25, 26]. To establish such a system, prior knowledge of attributes that critically affect quality of analytical results of the QC laboratory is important. Literature indicates that human factors, accommodation and environmental conditions, methods, equipments, sampling and sample preparations, and handling of analytical procedures are
some of the critical attributes [26-28]. Understanding these attributes and organizing them into a quality system can benefit a scientific risk-based approach. Even though information is scarce with regard to the application of such risk-based QbD approaches in pharmaceutical QC laboratories, there are indications of the usefulness of risk-based approaches to define analytical quality in clinical laboratory medicine [29, 30]. Therefore, this chapter reports for the first time the application of risk-based scientific QbD principles in the establishment of a pharmaceutical QC laboratory in a resource-limited setting, i.e. the Jimma University Laboratory of Drug Quality (JuLaDQ). In addition to the existing product and analytical QbD principles, laboratory QbD (lQbD) concept is introduced and will be thus developed using laboratory water as an important part.

2. METHODS

With the objective to contribute to the quality of medicines in Ethiopia and the Horn of Africa by QC analytical activities, the issue of establishing a pharmaceutical QC laboratory came into picture. Central strategic questions were first defined: (1) what is the purpose of the QC laboratory? (2) what standards are required? and (3) what are the laboratory user requirements? Laboratory quality attributes that must be considered during establishment and affect the performance of the established pharmaceutical QC laboratory were clearly defined and assessed. The laboratory quality attributes were then sorted out and criticality of each quality attribute was evaluated based on the existing set-up, the purpose of the QC-laboratory (provision of QC-analytical services) and the required standard (world health organization and if deemed required, supplemented with Ph. Eur. (EDQM) and USP standards). These attributes were considered to design the QC laboratory workflow, based upon which JuLaDQ was physically established and became a running pharmaceutical QC laboratory. Applying efficient control strategies for each of these attributes, a quality management system model based on WHO standards [31], supplemented with guidance from the international standards ISO/IEC17025: 2005 [32] was applied. Analogous to pQbD and aQbD, lQbD was thus defined and formally recognised, during the recent prequalification inspection by the WHO inspection team [33]. This chapter outlines the laboratory water system.

**Experimental**

**Materials and reagents**

Distilled water was obtained by distillation apparatus (W4000, Bibby Scientific, UK) while ultrapure water used as gradient solvent was obtained by a commercially available water purification system.
(Nanopure Analytical ultrapure water system, model number 7143, Thermofischer Scientific). HPLC grade acetonitrile (Fisher Scientific, UK) was used for gradient HPLC while all other chemicals used in this study were analytical grade and used as received.

**Procedures**

Practical experiments were conducted to evaluate different quality parameters with the objective of identifying the fit-for-purpose laboratory water for the QC analytical activities of JuLaDQ. Total chromatographic peak area was calculated for tap water, distilled water (before system cleaning), distilled water (after system cleaning) and ultrapure water from gradient high performance/pressure liquid chromatography (HPLC) analysis conducted using the following method: Agilent 1260 Infinity series HPLC, column: Purosphere C18 (4.0 mm x 250 mm, 5 μm, pore size 80 Å with guard column), mobile phase: gradient water/acetonitrile, gradient flow rate 2.0 ml/min, run time: 30 min and detection using diodearray detector (DAD) with quantification at 210 and 254 nm [34]. Detail of the gradient elution system is presented in Table 1.

<table>
<thead>
<tr>
<th>#</th>
<th>Time (min)</th>
<th>% Water</th>
<th>% Acetonitrile</th>
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<tr>
<td>1</td>
<td>0.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>21.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>30.0</td>
<td>0.0</td>
<td>100</td>
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</table>

UV-absorbance values for the four water grades was obtained using UV-Visible spectrophotometer (Celil instruments CE 7200, Cambridge, England) at wavelength of 210 and 254 nm. Similarly, conductivity (μS/cm) was tested using a multi-range conductivity meter (HI9033, Hanna instruments, Portugal) for the different water types produced in JuLaDQ. Moreover, different tests were conducted on tap water, distilled water and ultrapure water of JuLaDQ to evaluate their quality according to WHO water R quality requirements [35].

A pilot water R stability study was conducted using HPLC to set the time period for which water R could be used without compromising its quality. The water was stored in a 4-liter sodalime glass (type III) with black plastic closure (Wheaton, USA) standing upwards mimicking normal use, under room temperature (15-30°C). Gradient HPLC analysis was conducted on three consecutive days: day 0, day 1, and day 2.
3. RESULTS

The predefined purpose of the pharmaceutical QC laboratory to be established was production of reliable analytical QC results, which are essential to take correct decisions on medicines. Therefore, the design and establishment of the QC laboratory considered those factors which affect the quality of analytical results [25]. In line with this, JuLaDQ was established in Jimma University, Jimma, Ethiopia with the main objective to contribute to the quality of medicines in the Horn of Africa region by QC analytical activities (e.g. surveys, inspection-supporting, industrial/governmental release of medicines). To this end, JuLaDQ has implemented a quality management system, based on WHO, European GMP and ISO/IEC17025 (2005) GLP standards [31, 32, 36].

| Table 2. WHO quality requirements in QC laboratories as implemented in JuLaDQ [31]. |
|---------------------------------|---------------------------------|
| **Quality attributes**          | **Specifications**               |
| Personnul and organization      | Personnul Qualified, trained and experienced |
| Legal basis and organization    | Legal establishment and proper organizational structure |
| Sampling                        | Appropriate sampling plan and sample documentation |
| Samples                         | Samples unique identification and integrity during transport and storage |
| Test results                    | Appropriate monitoring and evaluation |
| Test reports                    | Include test results, and details of sample and test conditions |
| Records                         | Data integrity and availability |
| Methods                         | Proper validation |
| Equipment                       | Calibration, servicing and maintenance |
| Lab environment                 | Temperature and humidity monitoring and control |
| Documentation control           | Written standard operating procedures for each activity |
| Out-of-specifications           | Corrective and preventive actions |
| Customers                       | Complaint handling |
| Contracts                       | Supplier and sub-contractor management |
| Quality audits                  | Continuous internal and external quality audits |

The establishment of JuLaDQ applied the risk-based QbD principles. The target laboratory performance is compliance to quality standards set by WHO quality requirements to obtain the prequalification status. Similarly laboratory quality attributes were defined and analogous to pQbD and aQbD, the term lQbD is hence introduced. One example of the lQbD activity is the risk assessment, visualized by the Ishikawa (Fish-bone) diagram, used in the establishment of JuLaDQ (Figure 1).
Table 3. General laboratory water types and specifications according to different standards.

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<tr>
<td></td>
<td>Type 1</td>
<td>Type 2</td>
<td>Type 3</td>
<td>Type 4</td>
</tr>
<tr>
<td>Conductivity at 25 °C (μS/cm)</td>
<td>0.056</td>
<td>1.0</td>
<td>0.25</td>
<td>5.0</td>
</tr>
<tr>
<td>Resistivity at 25 °C (MΩ.cm)</td>
<td>18.0</td>
<td>1.0</td>
<td>4.0</td>
<td>0.2</td>
</tr>
<tr>
<td>TOC (μg/l)</td>
<td>100</td>
<td>50</td>
<td>200</td>
<td>NL</td>
</tr>
<tr>
<td>Sodium (μg/l)</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Chloride (μg/l)</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Silica (μg/l)</td>
<td>3</td>
<td>3</td>
<td>500</td>
<td>NL</td>
</tr>
<tr>
<td>Total solids (mg/l)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Bacteria (CFU/ml)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Endotoxins (IU/ml)</td>
<td>NS</td>
<td>NS</td>
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TOC: total organic carbon, NL: no limit, NS: not specified

*Prepared by distillation (= Distilled water)
*When bacteria levels need to be controlled, ASTM further classifies reagent grade water types into type A (bacteria: 1CFU/100 ml, endotoxins: 0.03 EU/ml), type B (bacteria: 10CFU/100 ml, endotoxins: 0.25 EU/ml), type C (bacteria: 1000 CFU/100).
*Water R: purified water in Ph. Eur. with additional specifications for heavy metals: 0.1 ppm, nitrates: 0.2 ppm, aluminium: 10 ppb, and ammonium: 0.2 ppm.
*The colour from solution prepared by addition of water R (10 ml) + dilute nitric acid R (1 ml) + silver nitrate solution R shows no change in appearance for at least 15 min.
*Other R-types described in the Ph. Eur. for special analytical purposes include: water, distilled (water R prepared by distillation); water, distilled, deionized (deionized water R prepared by distillation with a resistivity 18 MΩ.cm); water, ammonium-free; water, carbon dioxide-free (water R boiled for a few minutes and protected from the atmosphere during cooling and storage or deionized water R with resistivity 18 MΩ.cm); water, nitrate-free; and water, particle-free (water R filtered through a membrane with a pore size of 0.22 μm).
Figure 1. Ishikawa diagram for risk assessment in JuLaDQ laboratory.
Laboratory QbD workflow as applied to the establishment of JuLaDQ is presented in Figure 2. The overall laboratory quality attributes (LQA) affecting quality of analytical results (laboratory performance) were found to be laboratory design, environment, sample, method, personnel, equipment, consumables and quality control procedures. Accordingly, appropriate GLP/GMP is being maintained in JuLaDQ by implementing appropriate workflow of samples and test data according to the WHO standards (Table 2). Moreover, laboratory water was used as a typical but critical QbD-flow example (Figure 2) to demonstrate IQbD. Internationally recognised laboratory water quality standards define different types presented in Table 3 [37-40].

Since a single water purification unit operation process could not consistently and with sufficient robustness provide the Ph. Int. water R quality requirements, a customized water purification system combining different feasible and setting-suitable water purification processes including filtration, distillation, and ultrapure water purification technology (reverse osmosis (RO) and ultra-filtration) was set-up as presented in Figure 3.

<table>
<thead>
<tr>
<th>#</th>
<th>Test</th>
<th>Specification limit</th>
<th>Compliance (√)/Non-compliance (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heavy metals</td>
<td>Color not darker than the same untreated purified water</td>
<td>√</td>
</tr>
<tr>
<td>2</td>
<td>Ammonia</td>
<td>Color of test solution is not more intense than standard solution</td>
<td>√</td>
</tr>
<tr>
<td>3</td>
<td>Calcium and magnesium</td>
<td>Pure blue color</td>
<td>√</td>
</tr>
<tr>
<td>4</td>
<td>Chlorides</td>
<td>Clear and colorless</td>
<td>√</td>
</tr>
<tr>
<td>5</td>
<td>Nitrates</td>
<td>No blue color appeared at the interface of the two liquids</td>
<td>√</td>
</tr>
<tr>
<td>6</td>
<td>Sulfates</td>
<td>Clear and colorless</td>
<td>√</td>
</tr>
<tr>
<td>7</td>
<td>Oxidizable matter</td>
<td>Faintly pink test solution</td>
<td>√</td>
</tr>
<tr>
<td>8</td>
<td>Non volatile residue</td>
<td>&lt;0.001%</td>
<td>√</td>
</tr>
<tr>
<td>9</td>
<td>Alkalinity/acidity</td>
<td>No red color up on addition of methyl blue and no blue color appears up on addition of bromothymol blue</td>
<td>√</td>
</tr>
</tbody>
</table>

*After cleaning
The typical analytical quality results of the three water types produced in JuLaDQ (tap water, distilled water and purified water) according to Ph.Int water R quality requirements is presented in Table 4, while the overall analytical quality for the different water grades is presented in Table 5.

Figure 2. Laboratory QbD workflow and its application to laboratory water.
Table 5. Overall analytical quality of different water grades in JuLaDQ using different parameters.

<table>
<thead>
<tr>
<th>#</th>
<th>Water type</th>
<th>Typical chromatogram</th>
<th>Total peak area (mAU*s)</th>
<th>UV-absorbance (AU)</th>
<th>Conductivity (μS/cm) (resistivity in MΩ·cm)</th>
<th>Water R Ph. Int. compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>210 nm</td>
<td>254 nm</td>
<td>210 nm</td>
<td>254 nm</td>
</tr>
<tr>
<td>1</td>
<td>Tap water</td>
<td></td>
<td>92435</td>
<td>8520</td>
<td>0.623</td>
<td>0.111</td>
</tr>
<tr>
<td>2</td>
<td>Distilled water before cleaning</td>
<td></td>
<td>88206</td>
<td>7199</td>
<td>0.399</td>
<td>0.107</td>
</tr>
<tr>
<td>3</td>
<td>Distilled water after cleaning</td>
<td></td>
<td>43384</td>
<td>3551</td>
<td>0.317</td>
<td>0.097</td>
</tr>
<tr>
<td>4</td>
<td>Ultrapure water</td>
<td></td>
<td>722</td>
<td>59</td>
<td>0.098</td>
<td>0.054</td>
</tr>
</tbody>
</table>

NA: Not applicable
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The results of the pilot HPLC stability study for ultrapure water is presented in Table 6.

Table 6. Pilot HPLC stability results of ultrapure water for three consecutive days (sodaline glass (type III) container with black plastic closure, standing upwards mimicking normal use, under room temperature (15-30°C)).

<table>
<thead>
<tr>
<th>#</th>
<th>Experiment</th>
<th>Time (hr)</th>
<th>Total peak area (mAU*s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Day 0</td>
<td>0</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>Day 1</td>
<td>24</td>
<td>12.8</td>
</tr>
<tr>
<td>3</td>
<td>Day 2</td>
<td>48</td>
<td>68.7</td>
</tr>
</tbody>
</table>

Figure 3. Customized JuLaDQ water purification system.

4. DISCUSSION

An important point in designing a pharmaceutical QC laboratory for improved quality based on the QbD principles is defining causes of variability and devising appropriate control strategies in order to reduce the associated risks of laboratory performance. The cornerstone concepts in such IQbD principles are target laboratory profile (TLP), laboratory quality attributes (LQA), risk assessment, critical process parameters (CPPs), control strategy, and continuous improvement (Figure 1). Quality target profile (QTP) forms the basis of QbD, which is in relation to the predefined objective criteria. The concepts of analytical target profile (ATP) and Target Product Profile (TPP) described and defined in ICH Q8 [2] parallel IQbD’s target laboratory profile (TLP). TLP is therefore the prospective summary of the quality characteristics of a QC laboratory that ideally will be achieved to ensure the desired quality standard. For a QC-laboratory, it implies developing quality system based on regulatory requirement guidelines: good laboratory practices (GLP) and/or good manufacturing practices (GMP). TLP is compliance to the requirements of good practices for pharmaceutical quality.
control laboratories (GPQCLs) set by WHO [31] supported mainly by the international standards ISO/IEC 17025:2005 [32], which is the prime target for JuLaDQ.

High quality material and consumables are critical for efficient and precise laboratory performance. The quality of reagents can influence quality of test results. For example, reagent water quality affects nearly every aspect of HPLC analysis, from sample and standard preparation to column rinsing and elution. That makes water the largest reagent consumed in terms of volumes. Between 70% and 80% of HPLC performance problems are attributable directly to the quality of water used in preparing HPLC eluents, standards, and samples. Poor quality water reduces chromatographic performances by affecting resolution and integration, introducing ghost peaks, altering stationary phase selectivity, and impacting baselines [41]. Therefore, water R quality is critical for pharmaceutical QC laboratories performance.

Many analytical scientists consider water quality to be one of the critical factors that influence accuracy and reliability of the obtained analytical results. Water is used to prepare buffers, blanks, controls, sample solutions, and mobile phases in analytical procedures [42]; and using the appropriate water quality is of utmost importance in a resource-limited environment where the costs and handling also play a role. As our main objective is setting up a QC medicine laboratory according to WHO accepted standards, analytical water as defined in the Ph. Int. (water R) is our (minimum) quality target.

For accurate and reliable analytical results obtained from pharmaceutical QC laboratories, water R according to Ph. Int. is critical since Ph. Int. methods are/will be mostly used in JuLaDQ. The general quality attributes for laboratory water were listed down to be conductivity/resistivity, turbidity, microbial content, endotoxins and total organic carbon (TOC) [43-46]. However, TPP reveals that the product water should comply with water R requirements set in Ph. Int., and the LTP indicates that JuLaDQ is not meant to perform biological and/or microbiological tests; the CQAs for laboratory water are those attributes which are described in Ph. Int. Therefore, CQAs for laboratory water include heavy metals, ammonia, calcium and magnesium, carbon dioxide, chlorides, nitrates, sulphates, oxidizable matter, non-volatile residue and acidity or alkalinity [35].

To clearly define and identify critical water purification process parameters (CPPs), a number of practical experiments were conducted. The literature specifications for different water types (Table 3) revealed that no single water purification unit operation process could provide the TPP, analytical quality water R of the Ph.Int. Therefore, a customized water purification system combining different CPPs including filtration, distillation, and nanopure water purification technology was designed and installed (Figure 3).

Since biological and/or microbiological testing of medicines is currently not performed in JuLaDQ, microbial content and endotoxins tests were not defined to be current critical quality attributes.
The practical experimental results (Table 4) indicated that not only ultrapure water but also distilled water comply with the water R analytical quality specification set in Ph. Int. making cost estimation for production of both water types very demanding in such a resource-limited setting. Therefore, the QTP was evaluated not only towards the water quality target, but also towards operational cost (Table 7).

**Table 7.** Estimated cost per liter for production of water R (distilled and ultrapure water) in JuLaDQ.

<table>
<thead>
<tr>
<th>Water R (Ph. Int.)</th>
<th>Equipment name</th>
<th>Equipment cost (USD)</th>
<th>Electricity cost/l (USD)</th>
<th>Consumables/l</th>
<th>Operator/l</th>
<th>Subtotal cost/l (USD)</th>
<th>Total cost/l (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Merit Still, W4000 (Bibby Scientific, UK)</td>
<td>1,270.00</td>
<td>0.3</td>
<td>0.013</td>
<td>0.0001</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Ultra Pure water</td>
<td>Nanopure Analytical ultrapure water system, Model # 7143 (Thermo Scientific, USA)</td>
<td>8,500.00</td>
<td>1.7</td>
<td>0.0001</td>
<td>0.4</td>
<td>0.5</td>
<td>2.6</td>
</tr>
</tbody>
</table>

1Five years (250 weeks) depreciation and 20 l water/week was used for calculation.
2The total cost to produce 1 l ultrapure water includes cost of distilled water/l since distilled water is used as feed water.
3HPLC-gradient grade packed water/l costs 60 USD.

Cost for the production of both distilled and ultrapure water was estimated with the assumption that equipment depreciates after 5 years (about 250 weeks) with 20 l water consumption per week in the actual setting. Hence both equipment and operational cost per liter of water produced for each water R types was calculated providing the total cost per liter for each product. It is obvious that the cost of production of ultrapure water (3.2 USD/l), which also includes the cost for distilled water, is about five times higher than that of distilled water (0.6 USD/l). However, the water R quality specification set in Ph. Int. is the minimum requirement, and distilled water of JuLaDQ does not meet the quality requirements of water for chromatography set in Ph. Eur. since its resistivity (1.9 MΩ.cm) (Table 5) is by far less than the minimum resistivity requirement (≥ 18 MΩ.cm) (Table 3). Moreover, the cost of purchasing HPLC-grade packed purified water is 60 USD/l, which is unimaginable in such a resource-limited setting. Therefore, production of the ultrapure water (resistivity = 18.2 MΩ.cm) which complies with the resistivity requirement set in Ph. Eur. is very demanding, specially in the case of gradient HPLC systems.

In Ph. Int., most often only isocratic system is used for cost and ruggedness reasons. However, some analytical methodologies require gradient HPLC at longer retention times making the gradient system
more demanding even in resource-limited settings. Moreover, since in the future, we need to include endotoxin test, the ultrapure equipment is able to produce water for bacterial endotoxin test (BET) according to Ph. Int. Therefore, even though there was considerable variation in the resistivity values (MΩ.cm) between the two water types, and since resistivity is not a formal quality specification for water R in Ph. Int., it is possible to conclude that the ultrapure water (resistivity = 18.2 MΩ.cm) could be preserved for gradient HPLC experiments and the proposed future BET, while the distilled water can be utilized for isocratic HPLC analysis, glassware cleaning and rinsing analytical activities.

Standards and norms such as ASTM D1193 specify that water be drawn and used within 8 hr, which might not be practical in actual settings. Therefore, optimal time of use should be established and the pilot stability study results (Table 6) reveal that water R can be used for 48 hr without degrading in its quality if stored under normal conditions upwards in a sodalime glass (type III) container tightly covered with plastic stoppers.

In pQbD, control strategy is a planned set of controls derived from current product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to the product and inputs, facility and equipment operation conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control [18, 47]. In aQbD, control strategy includes the system suitability tests (SSTs) and revalidation aspects whenever need. Similarly, appropriate SSTs are employed as control element to ensure that consistent quality of water R according to Ph. Int. is maintained. Routine SSTs for water R are currently not included in Ph. Int., but essential to ensure consistent product quality. Moreover, all the quality parameters indicated in the Ph. Int. are qualitative colour reactions, and no quantitative specification limits are set. Therefore, our proposed SSTs are new elements in the routine control strategy for water R according to Ph. Int.

The experimental results with regard to the overall analytical quality evaluation of different water grades in JuLaDQ (Table 5) indicated that HPLC chromatograms (and the global peak area at wavelength of 210 and 254 nm) and conductivity/resistivity are parameters that have strong discriminatory effect between different water types than UV-absorbance. In the chromatograms, it is observed that there was rise in baseline, number and size of peaks obtained across ultrapure, distilled (after and before cleaning), and tap water. Quality parameters like the global peak area (mAU*s) at 210 and 254 nm, conductivity (μS/cm) (reverse for resistivity (MΩ.cm)) and UV-absorbance (AU) are increasing in similar fashion, but with different rates. For example, the ratio of HPLC chromatogram global peak area (mAU*s) at 254 nm for distilled water to ultrapure water was found to be 60.2 while the same ratio from the UV-absorbance (AU) was only 1.8. Similarly the ratio of resistivity (MΩ.cm) between tap and distilled water indicated very significant figure (967.3). Therefore, both HPLC
chromatogram global peak area (mAU*s) (at 210 and 254 nm) and resistivity (MΩ.cm) at 25 °C should be utilized as the routine SST parameters and need to be controlled.

Using the HPLC chromatograms global peak area (mAU*s) at 210 and 254 nm (Table 5), it is possible to effectively discriminate between different water qualities based on compliance to water R quality requirements set in Ph. Int. For example, the global peak area (mAU*s) at 254 nm for distilled water that complies with water R (Ph. Int.) was 3,551 while that of tap water that failed the water R quality requirement was 8,520. Therefore, it is very logical to propose a specification limit of 5,000 mAU*s for global peak area at 254 nm as an SST parameter. Similarly, we propose a specification limit of 5,500 mAU*s for the global peak area at 210 nm. These HPLC-UV SSTs are an alternative for TOC (total organic carbon), which requires additional and expensive equipment.

According to ASTM, water type 2 is produced by distillation and is similar to distilled water in JuLaDQ, which complies with water R in Ph. Int. The resistivity (MΩ.cm) for water type 2 (= distilled water) is ≥ 1.0, a value which can be taken as a routine SST specification for water R.

**Table 8.** Specifications for system suitability test parameters (Resistivity and HPLC/DAD global peak area) for water R of Ph. Int.

<table>
<thead>
<tr>
<th>#</th>
<th>System suitability test (SST)</th>
<th>Specification set for water R, Ph. Int.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resistivity at 25 °C (MΩ.cm)</td>
<td>≥ 1</td>
</tr>
<tr>
<td>2</td>
<td>HPLC chromatogram global peak area (mAU*s)</td>
<td>254 nm &lt; 5000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>210 nm &lt; 5500</td>
</tr>
</tbody>
</table>

*Proposed specifications which will be followed-up in the control strategy and adapted if required.

The proposed specification limits will be followed-up in the control strategy and adapted if required. Every month, a sample from each water R in JuLaDQ will be taken and subjected to full analysis (Ph. Int. tests, HPLC/DAD, UV-absorbance, conductivity/resistivity). Control chart will be constructed and the upper control limit (UCL) and lower control limit (LCL) will be calculated for each of the proposed SSTs parameter, and the currently proposed SST specification limits evaluated.

As such effective control strategy for water R quality is currently being implemented in JuLaDQ; continuous improvement fundamentally aims to reduce the common causes of variability over these SSTs parameters. For pharmaceutical QC laboratories, continuous improvement could be achieved through quality meetings (e.g. JuLaDQ has a formal and documented management review meeting once annually). This management review seeks to widely and properly evaluate comprehensive information from different sources, such as the audit results (WHO inspection report) [33], and propose adequate corrective actions to ensure continual improvement of the system [31, 32, 48].


5. CONCLUSIONS

Applying QbD principles, Jimma University Laboratory of Drug Quality (JuLaDQ) is established in Ethiopia with the goal of contributing to QC analytics of medicines in the Horn of Africa. Analogous to pQbD and aQbD, lQbD is proposed here and applied on the QbD-based establishment of laboratory water system. Target laboratory profile (TLP), laboratory quality attributes (LQA), laboratory quality risk assessment, control strategy and continuous improvement pertinent to lQbD were defined. Water of appropriate quality that has critical effect on the quality of analytical results is established in JuLaDQ so that the QC laboratory can effectively contribute to the intended purpose.

6. REFERENCES

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[40] European Pharmacopoeia 8.0 (Ph. Eur.). Water, purified. 2015


CHAPTER III

QUALITY ANALYTICS OF THE ANTIMALARIAL DRUG LUMEFANTRINE

“It is easy to get a thousand prescriptions but hard to get one single remedy.”

Chinese Proverb

Parts of this chapter were published:


ABSTRACT

Lumefantrine (benflumetol) is a fluorene derivative belonging to the aryl amino alcohol class of antimalarial drugs and is commercially available in fixed combination products with b-artemether. To better understand the quality analytics of lumefantrine, firstly HPLC-DAD/UV method of analysis was developed and HPLC-DAD/UV-ESI/ion trap/MS was applied for exhaustive impurity profiling of lumefantrine. Accordingly, a comprehensive impurity profile was established based upon analysis of market samples as well as stress, accelerated and long-term stability results. In-silico toxicological predictions for these lumefantrine related impurities were made using Toxtree® and Derek®. Several new impurities are identified, of which the desbenzylketo derivative (DBK) is proposed as a new specified degradant. DBK and the remaining unspecified lumefantrine related impurities are predicted, using Toxtree® and Derek®, to have a toxicity risk comparable to the toxicity risk of the API lumefantrine itself. Secondly, a simple gas chromatographic method coupled to flame ionization detector was developed and validated for quantitative determination of lumefantrine through derivatization technique using silylating agent N,O-bis(trimethylsilyl) trifluoro-acetamide. Evaluation of the method within analytical quality-by-design principles indicated that the method has acceptable specificity, accuracy, linearity, precision, and high sensitivity. The developed method was successfully applied to analyze the lumefantrine content of marketed fixed dose combination anti-malarial finished pharmaceutical products.
Main focus in this chapter:
- To develop and validate HPLC/DAD method
- To exhaustively profile the impurities of lumefantrine using HPLC/DAD/UV-ESI/Ion Trap/MS.
- To develop and validate a simple GC/FID method for lumefantrine determination in finished pharmaceutical products that can be utilized for routine regulatory quality control activities.

1. INTRODUCTION

Lumefantrine (benflumetol) is a 2,4,7,9-substituted fluorene (2,3-benzindene) derivative (Figure 1). It was synthesized in the 1970s by the Academy of Military Medical Sciences, in Beijing, and registered in China for anti-malarial use in 1987. It is now commercially available in fixed combination products, mostly with β-artemether (ACT, artemisinin-based combination therapy), which are proven to be highly efficacious for treatment of uncomplicated *falciparum* malaria. Subsequently, the compound proved to possess marked blood schizontocidal activity against a wide range of plasmodia, among them chloroquine-resistant *Plasmodium falciparum* [1-5].

![Figure 1. The chemical structure of lumefantrine.](image-url)
Biochemical studies suggest that its anti-malarial effect involves lysosomal trapping of the drug in the food vacuole of the intra-erythrocytic parasite, followed by binding to haem that is produced in the course of haemoglobin digestion. This binding prevents the polymerization of haem into haemozoin, hence inhibiting the detoxification of haem. Investigations involving aryl-methanol compounds have suggested the coordination of the iron centre of haem (Fe(III)PPIX) and related porphyrins by the alcohol functionality, indicating the structural activity relationship of the anti-malarial drug lumefantrine [6]. Hence, structural analogues of lumefantrine also possess marked anti-malarial effects. Halofantrine, an aryl amino alcohol analogue of lumefantrine, is also an anti-malarial drug but is known to cause cardiotoxicity [7]. Monodesbutyl-benflumetol, a metabolite of lumefantrine, exerts higher blood schizontocidal activity in *Plasmodium falciparum*, as well as in *P. vivax* where monodesbutyl-benflumetol is about 10 times more effective than lumefantrine [8]. The secondary alcohol permits the formation of dextrorotatory and levorotatory lumefantrine enantiomers and routine syntheses yield the racemate of (+)-lumefantrine and (-)-lumefantrine, which have almost identical potency. Therefore, from the activity point of view, there is no reason to use only one of the enantiomers of lumefantrine instead of the racemate. Moreover, in view of the low animal and human toxicity of lumefantrine racemate, no major toxicological differences between the two enantiomers are expected [9]. However, other synthesis impurities might be present.

Lumefantrine is incorporated in the WHO essential drug list for the treatment of malaria in endemic areas of the tropical climate. Due to the logistic system [10], degradation products may be spontaneously generated during distribution and storage. Control of such impurities in drug substances and finished drug products is required as they might impart different efficacy and bioavailability to the drug and/or they might produce different adverse and toxic effects to the patients [11].

The safety of a drug product is dependent not only on the toxicological properties of the active drug substance, but also on the toxicological properties of its impurities [12]. Thus, there is an ever increasing interest in impurities present in APIs and FPPs [13]. Impurity profiling (i.e. the identity as well as the quantity of impurities in the pharmaceutical drug) is now gaining critical attention from regulatory authorities. The different Pharmacopoeias, such as the European Pharmacopoeia (Ph.Eur.), United States Pharmacopeia (USP) and International Pharmacopoeia (Ph.Int.) are incorporating specification limits to allowable levels of impurities present in the API’s or FPPs formulations, based upon found levels in approved market samples [11, 14, 15]. Moreover, ICH guideline Q3A(R) stipulates different thresholds or action limits based upon the maximum daily dose (MDD). For lumefantrine formulations (FPP), with a MDD of 960 mg/day, these are defined as 0.10 % reporting threshold, 0.20 % identification threshold and 0.20 % qualification threshold [16].
USP-MC and Ph. Int. have already established specific limits for three lumefantrine related impurities: lumefantrine related compound A, lumefantrine related compound B, and lumefantrine related compound B. The USP-MC specification limits of these impurities are 0.1% for both impurities A and B, and 0.3% for impurity B. The Ph.Int. lumefantrine monograph lists the same three compounds as identified potential impurities, with specification limits of 0.1% for impurity A and 0.3% for impurity B.

Many methods have already been reported for the determination of lumefantrine in FPPs. HPLC methods are also reported for the simultaneous determination of lumefantrine and β-artemether in artemisinin-based anti-malarial FDC products. Micro-emulsion electrokinetic chromatography was developed as an alternative method to liquid chromatography for the determination of lumefantrine. However, no impurity profile has been established for this drug, while this is considered much more critical than the assay value. In this study, the potential impurities are described, including new degradants, as well as their relevance towards specification settings and in-silico toxicological evaluation. APIs and FPPs containing lumefantrine were evaluated by HPLC, with UV detection for quantification and with ESI-iontrap MS detection for identification.

Moreover, since there is no gas chromatographic (GC) assay method available, despite GC is a suitable technique in poor resource economies due to its ease of operation and maintenance, lower use-costs, and high separation efficiency. Therefore, this paper reports a GC-FID method for the quantitative determination of lumefantrine in anti-malarial FPPs using silylation with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

2. METHODS

Samples and chemicals
All drug substance batches (APIs), FPPs (Co-Artesian, Artesian, Lumartem and Coartem) and standard of desbenzylketo (DBK) lumefantrine derivative were supplied by Dafra Pharma International (Belgium), and were either synthesized in-house or purchased from commercial market. Standards of lumefantrine and impurity A USP-MC were purchased from U.S. Pharmacopeia (Basel, Switzerland). Analytical solutions were prepared in HPLC grade tetrahydrofuran (THF) (Fisher Scientific, Leicestershire, UK). Hydrogen peroxide (H₂O₂), sodium hydroxide (NaOH) and ammonium...
acetate were purchased from Merck (Darmstadt, Germany), hydrochloric acid (HCl) from Sigma-Aldrich (St Louis, USA) and glacial acetic acid from Riedel-de Haën (Seelze, Germany). Sartorius (Göttingen, Germany) ultrapure 18.2 MΩ.cm quality water and HPLC grade acetonitrile (Romil, Cambridge, UK) were used for HPLC-UV/MS analysis. Derivatization for GC analysis was performed using extra pure N,O-bis(trimethyl-silyl)trifluoro-acetamide (BSTFA) (Fisher Scientific, Leicestershire, UK).

HPLC-UV/MS impurity profiling

Liquid chromatography

HPLC-UV investigation of the impurity profiles was performed on a HPLC-PDA apparatus consisting of a Waters Alliance 2695 separation module and a Waters 2998 photodiode array detector with Empower 2 software for data acquisition (all Waters, Milford, MA, USA). For PDA detection, the UV spectrum was recorded at 190–400 nm. Quantification was performed at 266 nm. The positive ion ESI and the collision-induced dissociation mass spectra were obtained from the LC-UV/MS apparatus consisting of a Spectra System SN4000 interface, a Spectra System SCM1000 degasser, a Spectra System P1000XR pump, a Spectra System AS3000 autosampler, and a Finnigan LCQ Classic ion trap mass spectrometer in positive ion mode (all Thermo, San José, CA, USA), mass to charge range m/z 100 to m/z 2000 at unit resolution and with a peak width of 0.25 daltons/z, equipped with a Waters 2487 dual wavelength UV detector (Waters, Milford, MA, USA) and Xcalibur 2.0 software (Thermo) for data acquisition. ESI was conducted using a needle voltage of 4.5 kV. Nitrogen was used as the sheath and auxiliary gas with the heated capillary set at 250 °C. UV-detection was used for quantification (at 266 nm), while ESI-ion trap MS detection was used for identification.

Liquid chromatographic determination of impurities in lumefantrine samples was performed using a Purospher STAR RP-18 endcapped (150 × 4.6 mm, 5 µm particle size) column (Merck, Darmstadt, Germany) with guard column at 30 °C under isocratic conditions with a mobile phase consisting of ammonium acetate (pH 4.9; 0.1 M) and acetonitrile (10:90, v/v). The flow rate was set at 2.0 ml/min (minimal run time: 30 min.). The injection volume was 10 µl. Under these conditions, lumefantrine elutes at approximately 22 min. A system suitability test (SST) was established by a minimal resolution between lumefantrine and an in-situ prepared N-oxide by H2O2 treatment. Apparent number of theoretical plates for lumefantrine peak should be more than 8 x 10³, while it should be more than 2 x 10³ for N-oxide of lumefantrine.

The liquid chromatographic method was validated for the determination of lumefantrine and its related impurities. The selectivity of the developed chromatographic method was established by the separation of lumefantrine and its impurities. A correlation coefficient (r²) of 0.9998 for lumefantrine
(0.0006 to 0.01 mg/ml) and 1.0 for impurity A and DBK (0.001 to 0.1 mg/ml) demonstrated that the HPLC method is linear in the lower range. LOD/LOQ values for lumefantrine, DBK and impurity A were calculated (S/N = 3 resp. 10): 0.0042 mg/ml and 0.013 mg/ml for lumefantrine, 0.003 mg/ml and 0.009 mg/ml for DBK and 0.032 mg/ml and 0.107 mg/ml for impurity A. The analytical stability of lumefantrine, impurity A and DBK was confirmed over a storage period of 24 hours at 5°C, i.e. the sample compartment temperature.

**Forced degradation**

Forced degradation of lumefantrine API and FPP was performed under heat, light, acidic, alkaline and oxidative stress conditions. In heat stress studies, the FPP powder (one gram) was incubated at 40, 50 and 60°C for respectively four, three and two days. The placebo powder (one gram) was incubated for two days at 60°C. In light stress studies, the FPP and placebo powder (one gram) were subjected to UV (three days incubation) and VIS (seven days incubation) light. Finally, FPP and placebo were stressed by adding 10 ml of 1 M HCl (acidic), 1 M NaOH (alkaline) or 1 % H₂O₂ (oxidative) to one gram of the powder to be examined. Samples were incubated, up to eight days, at 5, 25, 40, 50 and 60°C. After the incubation, samples were neutralized using NaOH (acidic), HCl (alkaline) or Na₂S₂O₅ (oxidative), and the solvent evaporated using freeze-drying. Similar stress conditions as that of FPP and placebo powder were followed for API (equivalent to 1 gram of FPP).

Additionally, different batches of FPP were included in long-term (up to 24 months, 30°C, 70-75 % RH) and accelerated (up to 6 months, 40°C, 75 % RH) stability studies according to ICH stability guidelines [26].

All samples were dissolved in THF (18 mg/ml) and analyzed using HPLC-DAD/UV-ESI/MS.

**In-silico toxicological predictions**

To make *in-silico* toxicological predictions for lumefantrine and its identified related impurities, two sources of toxicological predictions were used: Derek® (12.0.0) for Windows developed by Lhasa Limited (Leeds, UK) and Toxtree® (v1.60) developed by Ideaconsult Ltd. (Sofia, Bulgaria). Derek® (12.0.0) for Windows is an expert knowledge base system, containing descriptions of molecular substructures which have been associated with toxic endpoints (structural alerts), that predicts whether a chemical is toxic in humans, other mammals and bacteria. The program applies structure-activity relationships ((Q)SARs) and other expert knowledge rules to derive a reasoned conclusion about the potential toxicity of the query chemical [27, 28]. Toxtree® (v1.60) is an open source application, which is able to estimate toxic hazards by applying a decision tree approach and making structure-based predictions for a number of toxicological endpoints using different modules. We have
generated hazard estimation using three Toxtree® modules: Cramer rules with extensions, Benigni/Bossa rulebase and structure alerts for the in-vivo micronucleus assay in rodents.

Gas chromatographic method

Gas chromatography (GC)

An Agilent 7820 GC system (Agilent technologies, Waldbronn, Germany) was used to perform the analysis with a liquid autosampler. Samples were introduced in a split/splitless injection port and detection was performed by means of flame ionization detector (FID). An HP-5 (30 m length x 0.32 mm id, 0.25 µm film thicknesses) column (Agilent technologies, Waldbronn, Germany) was used for separation. The output signal was recorded and processed using EZChrom Elite software.

The column oven was programmed with initial column oven temperature of 80°C for 1 min, and increased to 325°C at a rate of 10°C/min, holding 325°C for 9.5 min. The total run time was 35 min. The injector and detector temperatures were kept at 300°C and 340°C, respectively. Helium (Air Products) was used as carrier gas with a head pressure of 106.7 kPa resulting in an initial column flow of 3.2 ml/min and an average velocity of 50 cm/s. Helium was also used as make-up gas for the FID detector. The make-up gas flow was 25 ml/min, while for hydrogen and air the flow was 30 ml/min and 400 ml/min, respectively. The split ratio was set at 10:1 and a 4 mm i.d. deactivated open-glass tube liner, packed with fused silica wool was employed. Samples were injected by the instrument’s autosampler with injection volume of 1.0 µL and THF was used to rinse the syringe between injections.

Preparation of solutions

Preparation of lumefantrine standard solution

Lumefantrine standard solution was prepared at 100 µg/ml concentration in THF. 250.0 µl of this standard solution was transferred into a micro-vial and evaporated to dryness under nitrogen to obtain the final concentration of 500 µg/ml after derivatization.

Preparation of lumefantrine test sample solution

Four samples of fixed dose combination tablets (Coartem® and Artemine®) containing 120 mg of lumefantrine per tablet and one sample of powder for oral suspension (Co-artesiane®) containing 1080 mg of lumefantrine per bottle were analyzed using the developed GC-FID method. For this, a homogeneous FPP powder weight equivalent to 10.0 mg lumefantrine was accurately weighed and transferred to a 10.0 ml volumetric flask. THF was added, shaken for 5 min and diluted to volume using the same solvent. The mixture was centrifuged (3 minutes at 1914 g) and a test sample solution
was prepared at 100 μg/ml concentration (10 x dilution) in THF. 250.0 μl of this test sample solution was transferred into a micro-vial and evaporated to dryness under nitrogen to obtain the residue providing the final concentration of 500 μg/ml after derivatization.

**Preparation of lumefantrine impurity solutions**

Four different lumefantrine impurity [desbenzyl derivative (DB), lumefantrine USP-MC related impurity A (alcohol isomer), N-oxide-lumefantrine and desbenzylketo derivative (DBK)] solutions were prepared at 1 mg/ml concentration in tetrahydrofuran (THF) (Nicolas and Scholz, 1998). 25.0 μl of each of these lumefantrine impurity solutions was quantitatively transferred into a micro-vial and evaporated to dryness under liquid nitrogen to obtain the residue providing the final concentration of 500 μg/ml after derivatization. This solution can also be used as a system suitability solution as part of the control strategy of analytical QbD.

**Derivatization**

The BSTFA derivatives of standards and sample solutions were prepared from the dry residues obtained as described above by reacting with 50.0 μl BSTFA solutions in the air-tight glass vials at 70˚C for 30 min in an oven. The resulting solutions were cooled and injected into GC without removing any excess of the derivatizing agent.

For optimization and robustness evaluation of the sample derivatization process, a centralized composite face-centered (CCF) design with 11 runs including 3 center points was used evaluating the influence of incubation time (min) and temperature (˚C). A lumefantrine reference standard solution at 100% lc was prepared and analyzed using the different experimental CCF-conditions indicated in Supplementary information (Table S1). Peak area for the main lumefantrine peak and the quantitative presence of other peaks (with a reporting threshold of 0.1% with reference to the main peak) were evaluated as responses.

**Validation**

Validation of the method was performed based on the international conference on harmonization (ICH) guideline [29].

**Linearity of calibration curve**

From a stock solution containing 100 μg/ml lumefantrine in THF, different aliquots were transferred into a micro-vial and evaporated to dryness under liquid nitrogen to obtain the residue providing the final concentrations of 400, 450, 500 (100% lc), 550 and 600 μg/ml after derivatization. Calibration
curves for concentration versus peak area were plotted and the obtained data were subjected to linear regression analysis.

**Precision**
For intra-day precision, six sample solutions (n=6) were prepared at 500 μg/ml lumefantrine concentration after derivatization and analyzed using GC. Similarly, the inter-day precision was evaluated in three consecutive days (n=18). Lumefantrine concentrations were determined and relative standard deviations (RSD) calculated.

**Accuracy (Recovery test)**
Accuracy was tested by recovery experiments where lumefantrine reference solutions were added to a placebo sample at three levels: 75%, 100% and 125% lc. At each level, samples were prepared in duplicate and recovery percentage was calculated.

**Specificity**
Specificity of the method was evaluated by injecting lumefantrine reference standard solution and its impurity solutions [DB, USP-MC impurity A, N-oxide-lumefantrine and DBK], both separately and mixed.

**Limit of Detection (LoD) and Limit of Quantitation (LoQ)**
Standard solutions of lumefantrine were prepared by serial dilutions, ranging from 10 to 0.05 μg/ml concentration after derivatization, and injected onto the GC system. The LoD was defined as the concentration for which a signal-to-noise ratio (S/N) of 3 was obtained and LoQ was considered to be the concentration at which S/N was 10.

**Robustness**
For robustness evaluation, a Plackett-Burman (fractional factorial) experimental design (PBD) consisting of 11 runs, including 3 center points, was used investigating four factors: injector temperature (°C), final column temperature (°C), temperature gradient (°C/min) and pressure (kPa) (Modde version 8, Umetrics Inc, USA). Two test solutions [lumefantrine reference standard solution at 100% lc and a solution containing a mixture of lumefantrine (at 100% lc) and its related impurities (at 1% lc each)] were prepared and analysed using different experimental conditions by varying the different analytical parameters: injection temperature (290, 300, and 310 °C), final column temperature (320, 325 and 330 °C), temperature gradient (8, 10 and 12 °C/min) and pressure (102, 107 and 112 kPa). Chromatographic resolution [between lumefantrine peak and two related
impurities, N-oxide lumefantrine and USP-MC impurity A] \((R_s)\); retention time \((t_R)\), peak asymmetry \((A_s)\), peak area of lumefantrine and limit of detection \((LoD)\) for the two lumefantrine impurities (N-oxide lumefantrine and USP-MC impurity A) were evaluated under each condition.

3. RESULTS AND DISCUSSION

HPLC-UV/MS impurity profiling

HPLC analysis of lumefantrine containing samples

Lumefantrine API and FPPs were exposed to diverse stress conditions for different periods. Additionally, FPPs were put on long-term and accelerated stability studies as well according to ICH. FPP samples in the long-term stability study were kept for up to twenty-four months at 30°C /75 % RH. In the accelerated study, the stability conditions were adjusted and FPP samples were kept for up to six months at 40°C/75 % RH. The unstressed and stressed API samples, as well as the unstressed (release), accelerated, long-term and stressed FPP samples were analyzed with the validated HPLC method. Five synthesis and four stress related lumefantrine impurities have been observed in lumefantrine containing samples (Table 1). The relative retention time \((RRT)\), relative to lumefantrine, of these impurities was defined and normalized quantification was performed with a reporting threshold of 0.10 %. Maximal actually observed levels of lumefantrine related impurities in different samples under different conditions were obtained (Table 2). None of these lumefantrine related degradation-impurities were observed above the reporting threshold \((i.e. > 0.10 \%)\) in unstressed API and release \((T_0)\) FPP samples. However, these lumefantrine degradants were observed in stressed API and FPP samples, and in FPP stability studies. Compound 1, 2 and 3 were observed in oxidative stressed API samples. Three lumefantrine related impurities were observed in stressed FPP stability samples: compound 1 \((60°C, 1 \text{ M NaOH}, T_{2d})\), compound 3 \((60°C, 1 \% \text{ H}_2\text{O}_2, T_{2d})\) and compound 4 \((50°C, 1 \text{ M HCl}, T_{3d})\). Compound 3 and 4 were also detected in the accelerated \((40°C/75 \% \text{ RH}, T_{6m})\) and long-term stability studies. A typical UV chromatogram illustrating the separation of lumefantrine N-oxide, DBK, desbenzyl lumefantrine derivative and lumefantrine is given in Figure 2.
Table 1. Structural information for the observed and/or reported lumefantrine related impurities.

<table>
<thead>
<tr>
<th>#</th>
<th>Compound [formula, mono-isotopic mass]</th>
<th>Structure</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desbenzylketo N-oxide [C_{23}H_{27}NO_3Cl_2, MW 435.14]</td>
<td><img src="image1" alt="Structure Image" /></td>
<td>Alkaline stress, Oxidative stress</td>
</tr>
<tr>
<td>2</td>
<td>Lumefantrine (mono-)desbutyl derivative [C_{26}H_{24}NOCl_3, MW 473.09]</td>
<td><img src="image2" alt="Structure Image" /></td>
<td>Oxidative stress, Metabolite</td>
</tr>
<tr>
<td>3</td>
<td>Lumefantrine N-oxide [C_{30}H_{32}NO_2Cl_3, MW 543.15]</td>
<td><img src="image3" alt="Structure Image" /></td>
<td>Oxidative stress, Degradation</td>
</tr>
<tr>
<td>4</td>
<td>2,7-dichloro-4-[2-(di-n-butylamino)-1-hydroxyethyl]-9H-fluoren-9-one; Desbenzylketo derivative (DBK) [C_{23}H_{27}NO_3Cl_2, MW 419.14]</td>
<td><img src="image4" alt="Structure Image" /></td>
<td>Oxidative stress, Acidic Stress, Degradation</td>
</tr>
<tr>
<td>5</td>
<td>2-(di-n-butylamino)-1-[2,7-dichloro-9H-fluoren-4-yl]ethanol; Desbenzyl derivative [C_{23}H_{29}NOCl_2, 405.16]</td>
<td><img src="image5" alt="Structure Image" /></td>
<td>Synthesis</td>
</tr>
</tbody>
</table>
# Table 1. Structural information for the observed and/or reported lumefantrine related impurities (continued)

<table>
<thead>
<tr>
<th>#</th>
<th>Compound [formula, mono-isotopic mass]</th>
<th>Structure</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Synthesis impurity found in lumefantrine API; Lumefantrine oxide [C$<em>{30}$H$</em>{32}$NO$_2$Cl$_3$, MW 543.14]</td>
<td><img src="image1" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
<tr>
<td>7</td>
<td>Synthesis impurity found in lumefantrine API; Lumefantrine oxide [C$<em>{30}$H$</em>{32}$NO$_2$Cl$_3$, MW 543.14]</td>
<td><img src="image2" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
<tr>
<td>8</td>
<td>(RS,Z)-2-(Dibutylamino)-2-(2,7-dichloro-9-(4-chloro-benzylidene)-9H-fluoren-4-yl)ethanol (isomeric compound); Impurity A (Ph. Int. / USP Salmous) [C$<em>{44}$H$</em>{74}$Cl$_{6}$O$_2$, MW 797.39]</td>
<td><img src="image3" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
<tr>
<td>9</td>
<td>Synthesis impurity found in lumefantrine API; Lumefantrine oxide [C$<em>{30}$H$</em>{32}$NO$_2$Cl$_3$, MW 543.14]</td>
<td><img src="image4" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
<tr>
<td>10</td>
<td>(15S,3R,5R)-1,3-bis((EZ)-2,7-Dichloro-9-(4-chlorobenzyl-idene)-9H-fluoren-4-yl)-2,6-dioxabicyclo[3.1.0]hexane; Impurity B$<em>8$ (USP Salmous) [C$</em>{44}$H$_{46}$Cl$_6$O$_2$, 797.39]</td>
<td><img src="image5" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
</tbody>
</table>
Table 1. Structural information for the observed and/or reported lumefantrine related impurities (continued)

<table>
<thead>
<tr>
<th>#</th>
<th>Compound [formula, mono-isotopic mass]</th>
<th>Structure</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2-((EZ)-2,6-Dichloro-9H-fluoren-4-yl)-3'-(((EZ)-2,7-dichloro-9H-fluoren-4-yl)-2,2'-bioxirane; Impurity B&lt;sub&gt;6&lt;/sub&gt; (USP Salmous) [C&lt;sub&gt;44&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;Cl&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;, 797.39]</td>
<td><img src="image" alt="Structure" /></td>
<td>Synthesis</td>
</tr>
</tbody>
</table>

Table 2. Percentage maximum actual levels of lumefantrine related impurities observed (1).

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>API</th>
<th>Stressed</th>
<th>Release</th>
<th>FPP Accelerated</th>
<th>Stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desbenzylketo N-oxide</td>
<td></td>
<td>1.39</td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>2</td>
<td>Monodesbutyl derivative</td>
<td></td>
<td>0.56</td>
<td></td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lumefantrine N-oxide</td>
<td></td>
<td>0.12</td>
<td></td>
<td></td>
<td>0.86</td>
</tr>
<tr>
<td>4</td>
<td>Desbenzylketo derivative</td>
<td></td>
<td>0.34</td>
<td></td>
<td></td>
<td>4.26</td>
</tr>
<tr>
<td>5</td>
<td>Desbenzyl derivative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Lumefantrine oxide (RRT ~ 0.49)</td>
<td></td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Lumefantrine oxide (RRT ~ 0.52)</td>
<td></td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Impurity A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Lumefantrine oxide (RRT ~ 0.59)</td>
<td></td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Impurity B&lt;sub&gt;A&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Impurity B&lt;sub&gt;B&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) RT: reporting threshold = 0.10 %
Identification of lumefantrine impurities with LC-MS/MS

The observed lumefantrine impurity peaks (related to synthesis as well as degradation processes) in stressed or unstressed API and FPPs were identified using LC-MS/MS, with one of them investigated for the first time and proposed as a new specified lumefantrine related impurity. The desbutyl, desbenzyl and isomeric compound A derivatives are already known lumefantrine impurities. The analytical characteristics of the remaining unidentified lumefantrine related impurities were obtained by analysis of MS data: \( m/z \) values (Table 3), isotopic-distributions in mass spectra (Figure 2) and MS/MS (fragmentation pattern for structural identification).

<table>
<thead>
<tr>
<th>#</th>
<th>Compound</th>
<th>( t_R ) (min)</th>
<th>RRT</th>
<th>Most abundant ( m/z ) observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Desbenzylketo N-oxide</td>
<td>1.79</td>
<td>0.08</td>
<td>436.14</td>
</tr>
<tr>
<td>2</td>
<td>Monodesbutyl derivative</td>
<td>3.25</td>
<td>0.15</td>
<td>474.00</td>
</tr>
<tr>
<td>3</td>
<td>Lumefantrine N-oxide</td>
<td>3.96</td>
<td>0.17</td>
<td>544.08</td>
</tr>
<tr>
<td>4</td>
<td>Desbenzyketo derivative</td>
<td>7.41</td>
<td>0.33</td>
<td>420.13</td>
</tr>
<tr>
<td>5</td>
<td>Desbenzyl derivative</td>
<td>7.69</td>
<td>0.34</td>
<td>406.09</td>
</tr>
<tr>
<td>6</td>
<td>Lumefantrine oxide</td>
<td>10.96</td>
<td>0.49</td>
<td>544.12</td>
</tr>
<tr>
<td>7</td>
<td>Lumefantrine oxide</td>
<td>11.45</td>
<td>0.52</td>
<td>544.12</td>
</tr>
<tr>
<td>8</td>
<td>Impurity A</td>
<td>12.70</td>
<td>0.58</td>
<td>528.10</td>
</tr>
<tr>
<td>9</td>
<td>Lumefantrine oxide</td>
<td>12.97</td>
<td>0.59</td>
<td>544.12</td>
</tr>
<tr>
<td>L</td>
<td>Lumefantrine</td>
<td>22.28</td>
<td>1.00</td>
<td>528.10</td>
</tr>
</tbody>
</table>

(1) Retention time (min.); (2) Relative retention time

The mono-isotopic mass of lumefantrine \([(1RS)-2-(Dibutylamino)-1-[(Z)-2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]ethanol] was calculated to be 527.15. The mass spectrum of lumefantrine main peak indicated the most abundant ion at an \( m/z \)-ratio of 528.10, with an isotopic distribution corresponding to the three chlorine atoms in its structure (\(^{35}\)Cl at 75.77% and \(^{37}\)Cl at 24.23%). In the mass spectrum of compound 1 (RRT \( \sim \) 0.08), 436.14 is observed to be the most abundant \( m/z \). The isotopic distribution is suggestive for a compound possessing two chlorine atoms, and is identical to the isotopic distribution of compound 4. The most abundant \( m/z \) value for compound 4 is 420.13, with a molecular formula of \( \text{C}_{23}\text{H}_{27}\text{NO}_{2}\text{Cl}_{2} \), i.e. desbenzylketo derivative (DBK). This MS-derived structure was confirmed by chemical synthesis of a DBK reference and its IR and NMR spectroscopic structure confirmation. This DBK reference standard gave similar chromatographic retention characteristics as well as DAD-UV spectrum as degradant 4 found in the samples. Based on the observed \( m/z \) values of compound 1 and DBK, compound 1 has an additional oxide to its structure, and is thus assigned the N-oxide of DBK. The most abundant ion found for compound 2 was \( m/z \) 474.00. Its isotopic distribution is characteristic for a compound possessing three chlorines and the molecular formula \( \text{C}_{26}\text{H}_{28}\text{NO}_{3}\text{Cl}_{3} \), i.e. the monodesbutyl derivative. As this compound is more hydrophilic than lumefantrine, it elutes much earlier than lumefantrine.
Compound 3 was found in the oxidative stressed FPP samples. Its most abundant $m/z$ is 544.08, with an isotopic distribution corresponding to that of lumefantrine, giving the molecular formula $C_{30}H_{24}NO_2Cl_3$. Based on the observed $m/z$ values of compound 3 and lumefantrine, compound 3 has an additional oxide to its structure. MS/MS fragmentation spectra, by collision induced dissociation (CID, energy 100 eV), of compound 3 showed peaks at $m/z$ 526.12 (loss of H$_2$O), 470.10, 396.99, 380.95, 346.23, 305.58, 298.06 (loss of C$_{14}$H$_7$Cl$_2$) and 152.30.

**Figure 2.** UV chromatogram of a mixed sample illustrating lumefantrine N-oxide (3), DBK (4) and desbenzyl derivative lumefantrine (5) and lumefantrine (Lum).
This impurity was thus identified as lumefantrine N-oxide. Compounds 6, 7 and 9 gave identical mass spectra with the most abundant ion found at m/z 544.12. The isotopic distributions are characteristic for a compound containing three chlorines and a molecular formula C\textsubscript{30}H\textsubscript{32}NO\textsubscript{2}Cl\textsubscript{3}, i.e. oxides of lumefantrine. As these three impurities are eluting at different retention time, they are most probably isomeric compounds with an –OH function at different positions on the lumefantrine aromatic ring structure.

**Specified lumefantrine impurity DBK**

The lumefantrine related compound 4, DBK (RRT ~ 0.33), was not only formed in stress stability samples, but was also observed in accelerated and stressed stability samples of FPP. Moreover, DBK was found to be present in market samples at a concentration ranging between 0.03 % and 0.12 %, determined by area normalization. Subsequently, DBK was synthesized for further analytical characterization, including confirmation of its relative retention time (RRT) and determination of its relative response factor (RFF). The RRT and the RRF of DBK relative to lumefantrine were experimentally determined to be 0.33 and 2.87 respectively. The UV spectra recorded for lumefantrine and DBK showed the wavelength of maximum absorption to be higher for DBK (app. 266 nm) than for lumefantrine (app. 234 nm), due to the benzyl group being replaced by the keto function. This impurity was observed in oxidative and acidic stress degradation, as well as in the accelerated and long-term ICH stability studies, justifying this degradant to be classified as a specified degradant, which was up till now not yet reported.

**In-silico toxicological predictions of lumefantrine and its related impurities**

Using the knowledge-based expert systems Toxtree® and Derek®, general toxicological and carcinogenic alerts for lumefantrine, as well as for its related observed and already described impurities, have been investigated. Since DBK is a specified lumefantrine related compound, the toxicity profile of DBK is of paramount importance. Based on the Cramer rules with extensions, Toxtree® clearly predicted general toxicity risks (class III), and genotoxic alerts (polycyclic aromatic hydrocarbons, halogenated benzene and H-acceptor-path3-H-acceptor) for DBK, which are identical to the API lumefantrine itself. According to the toxicological concern (TTC), the daily dosage for compounds classified in class III should be below 90 µg/person (60 kg)/day to be validated as non toxic [30]. Hence, the TTC value of 90 µg on the MDD of 960 mg lumefantrine corresponds to a limit of 0.01 % (90 µg/960 mg), which is far below the levels actually found.

The toxicity profile by Derek® of DBK is defined by several general toxicity alerts which are similar to lumefantrine: hERG channel inhibition and α\textsubscript{2u}-globulin nephropathy [31] plus additional photo-toxicity and -allergenicity. However, Derek® did not trigger any genotoxicity or carcinogenicity for DBK.
The other lumefantrine related impurities were also predicted in Toxtree® to have a high general toxicity similar to lumefantrine itself (depicted Class III), based on the Cramer rules with extensions, and genotoxicity risks. Again, Derek® clearly indicated a limit toxicity profile for the majority of lumefantrine related impurities compared to lumefantrine (hERG channel inhibition, α₂µ-globulin nephropathy). Only impurity B₆ triggered additional toxicity alerts (carcinogenicity/ mutagenicity, chromosome damage, eye/skin irritation, developmental toxicity, skin sensitization), indicative for a non-toxic profile compared to lumefantrine itself.

**Gas Chromatographic method**

**Method development**

The analytical target profile (ATP) was to develop a stability-indicating quantitative assay for lumefantrine in FPPs that can be used in poor resource economies. GC-FID methodology is thus an appropriate technique. The quality target method profile includes that the method should be ICH-validatable within a GMP environment of a QC laboratory, including compliance with general pharmacopoeial chromatographic requirements like minimal resolution and maximal asymmetry.

As lumefantrine has a relatively high melting (128-132°C) and boiling point (642.5°C) at 760 mmHg [32, 33], and a free alcohol functional group in its structure (Figure 1) which affects the inherent volatility of the compound [34], direct GC analysis without derivatization was unsuccessful. Using silylation reactions [35, 36], the non-volatile and unstable (degrading at 200 - 300°C) lumefantrine molecule could however be successfully analyzed with GC. The widely available BSTFA was used as a derivatization reagent in our GC-FID method.

To develop the stability-indicating GC-FID assay for lumefantrine, different chromatographic factors were initially evaluated using a one-factor-at-a-time (OFAT) approach. These factors include injection port (temperatures from 150 to 400°C were tested) and oven program. In the final method, lumefantrine eluted at a retention time (tᵣ) of 26.0 min. Retention times and relative response factor (RRF), defined as the ratio of the response of the impurity and the API under identical chromatographic conditions [37], values for lumefantrine and its related impurities are presented in Table 4. All the lumefantrine related impurities (DB, USP-MC impurity A, N-oxide-lumefantrine and DBK) were eluting at different retention times without any interference with the lumefantrine main peak. The run time of analysis was 35 min. A typical chromatogram obtained on a mixture of lumefantrine API and its related impurities is presented in Figure 3.
Table 4. Retention time ($t_R$) and relative response factor (RRF) for lumefantrine and its related impurities using GC-FID analytics.

<table>
<thead>
<tr>
<th>Compound</th>
<th>DB</th>
<th>DBK</th>
<th>N-oxide lumefantrine</th>
<th>Lumefantrine</th>
<th>USP-MC impurity A</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_R$ (min)</td>
<td>20.14</td>
<td>20.54</td>
<td>23.78</td>
<td>26.04</td>
<td>26.25</td>
</tr>
<tr>
<td>RRF</td>
<td>0.97</td>
<td>0.56</td>
<td>0.57</td>
<td>1.00</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Figure 3. GC-FID chromatogram of BSTFA derivatized mixture of lumefantrine API ($t_R$ 26.0 min) and its related impurities solution: DB (20.1 min), DBK (20.5 min), N-oxide lumefantrine (three peaks at $t_R$ 23.8, 25.8 and 26.4 min) and USP-MC impurity A (26.3 min).

Validation

Linearity

A linear correlation (regression coefficient, $r^2 = 0.9986$) was found between the peak areas and the concentrations of lumefantrine, in the assayed range (80 to 120% lc).
**Precision**

The mean content (± standard deviation) of lumefantrine in the intra-day precision analysis \( (n=6) \) was 96.1% lc ± 0.9% (RSD = 0.9%) while that of the inter-day precision analysis \( (n=18) \) was 96.3% lc ± 0.8% (RSD = 0.9%). The intra-day and inter-day precision %RSD values were lower than 2.0 %, demonstrating appropriate precision of the method [38].

**Accuracy (recovery test)**

The recovery test was performed by analyzing a spiked placebo. Lumefantrine mean recovery was 99.5% (RSD = 1.0%), indicating the accuracy of the method.

**Specificity**

The chromatogram obtained on the mixture of lumefantrine API and its related impurities (Figure 3) showed no related impurity peak interference with the main peak, proving the method can be used for the quantification of lumefantrine in the presence of its related impurities including its possible degradation products. Moreover, in the analyzed placebo samples, we did not observe any interfering peak from the excipients with the main peak.

**Robustness of derivatization procedure**

The optimum derivatization conditions were set up to BSTFA solution in the air-tight glass vials at 70 °C for 30 min in an oven. A CCF design was applied to optimize the sample derivatization process. Two factors, incubation time (from 20 to 40 min) and incubation temperature (from 60 to 80°C), that affect yield of derivatization were considered. The factor levels are indicated in Supplementary information (Table S2). Maximization of peak area of the derivatized analyte was the target of the optimization process. None of the regression coefficients (with the 95% confidence interval) for the linear and quadratic effects of incubation time and incubation temperature and the product of the time and temperature differed significantly from zero. Therefore, the effect of both variables and their product on peak area is considered not significant at 95% CI. Optimal and most robust conditions were assigned to the mid points (0-level), i.e. incubation temperature of 70 °C and incubation time of 30 min.

Moreover, there was no other peak observed above the reporting threshold of 0.1%, indicating that the derivatization mixture was stable and pure within the method operable design region (MODR), defined as 70°C ± 10°C and 30 min ± 10 min.
Robustness of chromatography

Plackett-Burman design (PBD), a two-level fractional factorial design, was used to test the robustness of the chromatographic part of the method. PBD is selected for robustness evaluation since it combines less experimentation with maximal information acquisition in the most efficient way. Four factors, with deliberate small deviations from the method settings, were considered: injection temperature (from 290 to 310 °C), final column temperature (from 320 to 330 °C), temperature gradient (from 8 to 12˚C/min) and pressure (from 102 to 112 kPa).

The contour plots of these chromatographic factors for lumefantrine peak resolution ($R_s$) from N-oxide lumefantrine is presented in Figure 4 (a-f). All $R_s$ results from both N-oxide lumefantrine and lumefantrine related compound A were greater than 1.5 revealing that the small deviations introduced in the four method parameters did not have a significant effect on the minimal $R_s$ specification set in Ph. Eur., which was defined as a critical method attribute [39, 40]. The retention time ($t_R$) for DBK was 20.54 min while that of lumefantrine main peak was 26.04 min indicating a clear and non-critical separation of these two peaks.

The injection temperature, final column temperature and pressure did not have a statistically significant effect on the retention time of lumefantrine at 95% CI although the retention time was increasing from 24.6 to 27.0 min with the decrease in the final column temperature from the (+) level (330 °C) to the (-) level (320 °C) and the same was true for pressure. Increase in temperature gradient from 8 to 12˚C/min led to the decrease in $t_R$ from 31.3 to 22.6 min.

The effect of the deviations of the four chromatographic parameters from the method setting on peak area, peak asymmetry ($A_s$) and limit of detection (LoD) was also evaluated and the effect of these four parameters was not significant at 95% CI. Moreover, all the results of the peak asymmetry $A_s$ comply with the set specification in Ph. Eur. [39].

Therefore, the deviations from the target method setting for the four parameters: injection temperature, final column temperature, temperature gradient and pressure did not affect the chromatographic parameter specifications revealing the robustness of the developed gas chromatographic method.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

LoD and LoQ of lumefantrine were estimated based on signal-to-noise ratio. According to the determined signal-to-noise ratio, the LoD and LoQ for lumefantrine were calculated to be 0.01 µg/ml and 0.04 µg/ml, respectively, indicating the sensitivity of the method.
Analysis of marketed FDC products

Table 5 gives the assay results of marketed samples obtained in Ethiopia. The lumefantrine content varied from 96.2% to 98.3% lc, within the 90 - 110% lc specifications [32]. The results were found to be comparable to the assay results obtained on the same samples using fused-core HPLC method, 97.9% to 101.5% lc [23].
### Table 5. Contents of lumefantrine in fixed dose combination (FDC) products (n=6 for each).

<table>
<thead>
<tr>
<th>FDC samples</th>
<th>Batch/Lot No.</th>
<th>lumefantrine mean content ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemine® tablets</td>
<td>7711</td>
<td>96.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>7976</td>
<td>96.6 ± 1.0</td>
</tr>
<tr>
<td>Coartem® tablets</td>
<td>F2010</td>
<td>96.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>F2006</td>
<td>96.4 ± 0.9</td>
</tr>
<tr>
<td>Co-Artesiane powder for oral</td>
<td>20460</td>
<td>98.3 ± 0.7</td>
</tr>
<tr>
<td>suspension</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD = standard deviation

### 4. CONCLUSION

Firstly, an exhaustive impurity profiling of lumefantrine was performed using HPLC-UV/ESI-ion trap MS. From unstressed, stressed and accelerated stability samples of lumefantrine API and FPPs, nine compounds were detected and characterized to be lumefantrine related impurities. One new lumefantrine related compound, DBK, was identified and characterized as a specified degradation impurity of lumefantrine in real market samples (FPPs). The in-silico toxicological investigation (Toxtree® and Derek®) indicated overall a lesser toxicity for the specified impurity DBK compared to the API lumefantrine itself.

Secondly, a GC-FID method for lumefantrine assay in pharmaceutical preparations was developed and validated within an analytical QbD approach. The method is linear, precise and sensitive. It makes use of simple sample preparation procedures and is not solvent consuming. The retention time of lumefantrine was 26.0 min and there was no interference from its related synthesis and degradation impurities and excipients. The developed method was successfully applied to analyze lumefantrine content in different marketed anti-malarial FPPs and can thus be applied to routine quality control of lumefantrine in pharmaceutical preparations.

### 5. REFERENCES


[38] Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis: understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *J. Chromatogr. A* 2003; **987**: 57–66.


## SUPPLEMENTARY INFORMATION

**Table S1.** Central composite face-centered (CCF) design experimental conditions and responses for sample derivatization procedure.

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Incubation temperature (°C)</th>
<th>Incubation time (min)</th>
<th>Lumefantrine peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>20</td>
<td>4723313</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>20</td>
<td>4835205</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>40</td>
<td>4870620</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>40</td>
<td>4899217</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>30</td>
<td>4775814</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>30</td>
<td>4711999</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>20</td>
<td>4934172</td>
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<td>4796128</td>
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<tr>
<td>11</td>
<td>70</td>
<td>30</td>
<td>4628999</td>
</tr>
</tbody>
</table>

**Table S2:** Plackett-Burman design: experimental conditions and response results for GC robustness evaluation.

<table>
<thead>
<tr>
<th>#</th>
<th>Injection T (°C)</th>
<th>Final column T (°C)</th>
<th>T gradient (°C/min)</th>
<th>P (kPa)</th>
<th>R&lt;sub&gt;L&lt;/sub&gt; from N-oxide Lum</th>
<th>R&lt;sub&gt;L&lt;/sub&gt; from USP-MC impurity A</th>
<th>Lum t&lt;sub&gt;R&lt;/sub&gt;</th>
<th>Lum A&lt;sub&gt;s&lt;/sub&gt;</th>
<th>LoD, N-oxide lum</th>
<th>LoD, USP-MC impurity A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>310</td>
<td>320</td>
<td>12</td>
<td>102</td>
<td>1.8</td>
<td>1.7</td>
<td>22.9</td>
<td>0.8</td>
<td>0.129</td>
<td>0.430</td>
</tr>
<tr>
<td>2</td>
<td>310</td>
<td>330</td>
<td>8</td>
<td>102</td>
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<td>2.0</td>
<td>31.5</td>
<td>0.8</td>
<td>0.081</td>
<td>0.269</td>
</tr>
<tr>
<td>3</td>
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<td>330</td>
<td>8</td>
<td>112</td>
<td>2.0</td>
<td>1.8</td>
<td>31.1</td>
<td>0.8</td>
<td>0.075</td>
<td>0.251</td>
</tr>
<tr>
<td>4</td>
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<td>330</td>
<td>12</td>
<td>112</td>
<td>1.8</td>
<td>1.8</td>
<td>22.3</td>
<td>0.8</td>
<td>0.050</td>
<td>0.168</td>
</tr>
<tr>
<td>5</td>
<td>310</td>
<td>320</td>
<td>12</td>
<td>112</td>
<td>1.9</td>
<td>1.8</td>
<td>22.5</td>
<td>0.8</td>
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<td>0.271</td>
</tr>
<tr>
<td>6</td>
<td>290</td>
<td>330</td>
<td>12</td>
<td>102</td>
<td>1.8</td>
<td>1.7</td>
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<td>2.1</td>
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<td>31.1</td>
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<td>0.061</td>
<td>0.202</td>
</tr>
<tr>
<td>8</td>
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<td>102</td>
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<td>1.9</td>
<td>31.5</td>
<td>0.8</td>
<td>0.080</td>
<td>0.266</td>
</tr>
<tr>
<td>9</td>
<td>300</td>
<td>325</td>
<td>10</td>
<td>107</td>
<td>1.9</td>
<td>1.9</td>
<td>26.1</td>
<td>0.7</td>
<td>0.047</td>
<td>0.158</td>
</tr>
<tr>
<td>10</td>
<td>300</td>
<td>325</td>
<td>10</td>
<td>107</td>
<td>1.9</td>
<td>2.0</td>
<td>26.1</td>
<td>0.8</td>
<td>0.050</td>
<td>0.168</td>
</tr>
<tr>
<td>11</td>
<td>300</td>
<td>325</td>
<td>10</td>
<td>107</td>
<td>1.9</td>
<td>1.9</td>
<td>26.1</td>
<td>0.8</td>
<td>0.054</td>
<td>0.180</td>
</tr>
</tbody>
</table>

T: temperature; P: pressure; Lum: lumefantrine.
“If Drug B costs 70 percent of Drug A, but has a side effect profile that brings every second patient to the hospital for a four-day admission, then it does not have value”

Derek Raghavan
(Australian medical oncologist)

Parts of this chapter were published:


ABSTRACT

Malaria is one of the most serious infectious disease and great public health problem caused by Plasmodium parasite infection predominating in Africa. Because of high efficacy and the ability to limit development of resistance, fixed dose combination of β-artemether and lumefantrine has been used as the first line malaria treatment in most malaria-endemic countries like Ethiopia. However, the current artemisinin FDC products like β-artemether and lumefantrine are inherently unstable and require controlled distribution and storage conditions, which are not always available in resource limited settings. Moreover, quality control is hampered by lack of suitable analytical methods. Therefore, there is a need for a rapid and simple, but stability-indicating method utilized for routine regulatory quality control of the products assisted by surveys for sustainable monitoring of their quality in the actual supply chains. For this, a rapid, robust, precise and accurate stability-indicating quantitative fused-core isocratic HPLC method was developed for simultaneous assay of β-artemether and lumefantrine. Moreover, quality survey for artemether-based FDC anti-malarial products was conducted in Jimma zone using visual inspection for packaging and labelling, identification, mass uniformity and assay as critical quality attributes. The results revealed compliance for all the investigated quality attributes with the acceptance criteria set in Ph. Int.
CHAPTER IV

QUALITY OF ARTEMETHER-BASED ANTIMALARIAL MEDICINES IN ETHIOPIA

Main focus in this chapter:
- To develop a rapid, robust, precise and accurate stability-indicating quantitative fused-core isocratic HPLC method that can be used for routine regulatory QC of β-artemether and lumefantrine.
- To give an overview of the quality of artemether-based antimalarial products in Ethiopia.

1. INTRODUCTION

Malaria is the most serious infectious disease of great public health concern endemic throughout most of the tropics and subtropics. In 2013, there is a total of 104 countries and territories in which malaria is endemic. Globally, an estimated 3.4 billion people are at risk of malaria. Approximately 243 million people annually develop symptomatic malaria [1, 2]. Most of these can be attributed to *Plasmodium falciparum*, but *P. vivax* and *P. knowlesi* can also cause severe diseases. The populations living in sub-Saharan Africa have the highest risk of acquiring malaria, and children under five years of age and pregnant women are the most severely affected [2, 3]. Malaria case management remains a vital component of malaria control strategies. This entails early diagnosis and prompt treatment with effective anti-malarial medicines [4]. World Health Organization (WHO) has recommended that all anti-malarials should consist of a combination of an artemisinin derivative with a co-drug such as lumefantrine, amodiaquine or mefloquine; most malaria endemic countries have now adopted artemisinin-based anti-malarial combination therapy (ACT) as first-line treatment of *P. falciparum* malaria in place of chloroquine, quinine and sulphadoxine-pyrimethamine fixed dose combinations [5]. However, the emergence of resistance is of great concern [6-8], and this problem is fuelled by poor quality anti-malarial drugs. Poor quality anti-malarials are a severe under-recognized public health problem, reducing the effectiveness of these drugs and threatening current treatment policies [9]. There are three main
types of poor quality medicines: substandard, degraded and counterfeit. Substandard drugs are produced with inadequate attention to good manufacturing practices and may have content outside accepted limits. The term counterfeit refers to medicines which are deliberately and fraudulently mislabeled with respect to identity and/or source and substandard medicines are genuine medicines produced by authorized manufacturers but do not meet the quality requirements, incl. GMP and QC-results, set for them by national standards [10-13]. Degraded formulations may result from (unwanted) exposure of initially well produced, good quality medicines to light, heat and humidity [9, 14]. Therefore, the ultimate purpose of stability testing is to provide evidence on how the quality of a drug varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommendations of storage conditions, retest periods and shelf life to be established. The two main chemical aspects of the drug product that play an important role in shelf-life determinations are the assay of active drug (efficacy) and degradants generated during the stability study (safety). The assay of drug product in stability test samples obviously needs to be determined using a stability-indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines [15, 16]. Moreover, the intrinsic stability of a finished drug product should also be considered as a possible quality attribute when evaluating and comparing different drug products with the same active pharmaceutical ingredient (API). For example, it has been demonstrated that the half-life of β-artemether-containing products at 50°C can range between 0.70 and 9.52 months [17].

β-artemether is a methyl ether derivative of artemisinin, which is a peroxide lactone isolated from the Chinese anti-malarial plant *Artemisia annua* (Figure 1). Chemically, it is (+)-(3-beta,5a-beta,6-beta,8a-beta,9-alpha,12-beta,12aR)-decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyrano(4,3-j)-1,2-benzodioxepin [18].

![Chemical structure of β-artemether.](image)

Lumefantrine (benflumetol) is a 2,4,7,9-substituted fluorene (2,3-benzindene) derivative. Chemically, it is (9Z)-2,7-dichloro-9-[(4-chlorophenyl)methylene]-a-[dibutylamino] methyl]-9H-fluorene-4-
methanol [19]. Both compounds are now commercially available in fixed combination products (ACT), which are proven to be highly efficacious for treatment of uncomplicated *P falciparum* malaria. The increasing use of these β-artemether-lumefantrine combination anti-malarial products and the intrinsic stability of these products require controlled storage conditions. However, in resource-limited settings, stability of these products is not guaranteed since the supply chains do not have consistently appropriate temperature and humidity quality assurance systems [20].

Therefore, there should be methods of detecting poor quality medicines. Currently, there are HPLC methods for the assay of β-artemether in finished pharmaceutical products (FPP) [21-23], as well as for lumefantrine analysis [24-26]. Only a few HPLC methods were reported for the quantitative determination of β-artemether and lumefantrine in fixed combination anti-malarial products [27-29]. However, no simple, rapid and proven stability-indicating method has been reported for the simultaneous analysis of both active pharmaceutical drug products. Hence, the study presented in this chapter reports a rapid, economical, precise and accurate method for the assay of β-artemether and lumefantrine in the presence of their degradants.

Official policy discourages the use of artemisinin monotherapies and other drugs, such as sulfadoxine-pyrimethamine, that are no longer effective against malaria and ACTs are free of charge for all age groups at public sector in Ethiopia [30, 31]. However, a grossly neglected aspect of malaria control is the importance that patients should not just have access to medicines, but should have access to good quality-assured medicines; which was emphasized in the resolution adopted by the United Nations Human Rights Council [32]. The consequences of using poor quality medicines range from prolonged sickness, treatment failure or variability, side effects, loss of income, increased healthcare costs to death. In addition, the society may lose confidence in otherwise effective medicines, in healthcare systems and suffer major economic losses [33, 34]. Of particular current relevance, poor quality anti-malarials containing subtherapeutic amounts of artemisinin derivatives or only one of the two active ingredients in ACT, the primary treatment recommended for uncomplicated falciparum malaria, are very likely to contribute to disastrous anti-malarial artemisinin resistance [35, 36], increasing mortality and morbidity and risking the loss of these vital medicines for malaria control [37].

Medicines quality evaluation studies are primarily important to provide information on the drug content and identify the cause (if any) of poor quality medicines circulating in the country. However, there is little existing knowledge about quality of medicines in general and anti-malarial drugs in particular in Ethiopia. This QC study reported in this chapter was, therefore, carried out to assess the regulatory quality of fixed-dose combination (FDC) ACT anti-malarial medicines circulating in Jimma zone, southwest Ethiopia.
2. MATERIALS AND METHODS

HPLC method development and validation

Samples and chemicals

β-artemether and lumefantrine APIs, Co-Artesiane® FPP powder for oral suspension, dihydroartemisinin (DHA), artemisinin, 9,10-anhydroartemisinin (AHA; late eluting impurity (LEI)) and α-artemether standards were supplied by Dafra Pharma International (Belgium). Coartem® and Artemine® samples were collected from different markets in Ethiopia. Analytical solutions were prepared using HPLC grade unstabilized tetrahydrofuran (THF) (Fisher Scientific, Leicestershire, UK) obtaining a concentration of 0.2 mg/ml β-artemether and 1.2 mg/ml lumefantrine corresponding to 100% label claim (lc). Hydrogen peroxide (H$_2$O$_2$) and sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany), hydrochloric acid (HCl) and o-phosphoric acid from Sigma-Aldrich (St Louis, USA). Sartorius (Göttingen, Germany) ultrapure 18.2 mΩ.cm quality water and HPLC grade acetonitrile (Fisher Scientific, UK) were used for HPLC-UV analysis.

Liquid chromatography and analytical conditions

The HPLC analyses were carried out using HPLC-PDA apparatus consisting of a Waters Alliance 2695 separation module and a Waters 2998 photodiode array detector with Empower 2 software for data acquisition (all Waters, Milford, MA, USA). The following fused-core stationary-phase chemistries were evaluated: (i) Halo RP-Amide (50×4.6 mm id; 2.7 µm particle size); (ii) Halo C18 (50×4.6 mm id; 2.7 µm particle size); and, (iii) Halo Phenyl-hexyl (50×4.6 mm id; 2.7 µm particle size), all Achrom (Machelen, Belgium) and all thermostated at 30 °C. Detection was performed from 190-400 nm. Lumefantrine quantification was done at 335 nm, a wavelength at which β-artemether and its related impurities are not absorbing. For β-artemether, quantification was performed at 210 nm. As N-oxide-lumefantrine might interfere if present, back-calculating the peak area of N-oxide-lumefantrine using peak area conversion factor from 335 nm to 210 nm (1.64) was performed and the obtained value was subtracted from the peak area at 210 nm. The injection volume was 3 µl. Isocratic mobile phases containing acetonitrile and 1 mM phosphate buffer pH 3.0 were used at a flow of 1.0 ml/min. The separation of β-artemether and lumefantrine was evaluated using different proportions of these mobile phase solvents and, for each condition, the retention factor (k') and asymmetry factor (A$_{as}$) were calculated based on the method described in European Pharmacopoeia (Ph. Eur.) 2.2.46 [38]. The optimal condition was achieved using the Halo RP-Amide column and a mobile phase composed of acetonitrile and 1 mM phosphate buffer pH 3.0 (52:48 V/V).
Preparation of solutions

Preparation of β-artemether and lumefantrine standard solution
Approximately 20.0 mg β-artemether and 120.0 mg lumefantrine reference standards were accurately weighed and transferred to a 100.0 ml volumetric flask. Eighty ml tetrahydrofuran was added to dissolve both compounds and the solution was diluted to volume using mobile phase.

Preparation of test sample solutions
Four samples of fixed dose combination tablets (Coartem® and Artemine®) containing 20 mg β-artemether and 120 mg lumefantrine and three powders for oral suspension stability samples (Coartesiane®) containing 180 mg β-artemether and 1080 mg lumefantrine were analysed using the validated fused-core HPLC method. For this, a homogenous FPP powder amount equivalent to 20.0 mg β-artemether and 120.0 mg lumefantrine was accurately weighed and transferred to a 100.0 ml volumetric flask. Eighty ml tetrahydrofuran was added, shaken for 5 min and diluted to volume using mobile phase. The mixture was filtered through 0.45 μm HPLC syringe filters and analysed using HPLC.

Preparation of stress solutions
Preparation of oxidative degradation of lumefantrine API solution
Approximately 120.0 mg lumefantrine API was accurately weighed and transferred into 100 ml Erlenmeyer flask; 45.0 ml tetrahydrofuran was added to ensure complete dissolution of lumefantrine and then 5.0 ml 30% hydrogen peroxide was added. The solution was boiled for 120 min under constant reflux and analysed using HPLC.

Preparation of acidic degradation of lumefantrine solution
Approximately 120.0 mg of lumefantrine API was accurately weighed into 100 ml Erlenmeyer flask; 10.0 ml of 1 M hydrochloric acid solution was added and incubated at 70ºC for 30 hours. Subsequently, the solution was neutralized by addition of 2.0 ml of 5 M sodium hydroxide solution and then 38.0 ml of THF was added. The mixture was sonicated for 5 min, filtered and analysed.

Preparation of heat stressed β-artemether API solution
Preparation of heat stressed β-artemether was performed as described by De Spiegeleer et al [23]. Briefly, approximately 20.0 mg of β-artemether API was accurately weighed and transferred into a glass HPLC vial. The vial was put in a heating block at 145ºC for 30 min, resulting in approximately 70% conversion of β-artemether to related degradation products. Then 1.0 ml tetrahydrofuran was added and the solution was quantitatively transferred to a 50.0 ml volumetric flask by addition of 40 ml tetrahydrofuran. The solution was then diluted to volume using mobile phase.
Validation

Linearity

A stock solution containing 250 µg/ml β-artemether and 1,500 µg/ml lumefantrine in THF was prepared in triplicate. Different aliquots of these solutions were diluted in a dilution solvent consisting of THF/mobile phase (80:20 V/V) to five different concentrations, corresponding to 160, 180, 200, 220 and 240 µg/ml of β-artemether, and 960, 1,080, 1,200, 1,320 and 1,440 µg/ml of lumefantrine. Calibration curves for concentration versus peak area were plotted for each compound and the obtained data were subjected to linear regression analysis.

Precision

For intra-day precision, six sample solutions (n=6) were prepared at 0.2 mg/ml β-artemether and 1.2 mg/ml lumefantrine concentrations and analysed using HPLC. Similarly, the inter-day precision was evaluated in three consecutive days (n=3×6). β-artemether and lumefantrine concentrations were determined and relative standard deviations (RSD) were calculated.

Accuracy (recovery test)

Accuracy was tested by recovery experiments where β-artemether and lumefantrine reference solutions were added to a placebo sample at three levels: 75%, 100% and 125% of the label claim. At each level, samples were prepared in duplicate and recovery percentage was calculated.

Selectivity

Selectivity of the method was evaluated by injecting the stressed β-artemether and lumefantrine solutions as well as reference standard solutions of α-artemether, artemisinin, DHA and AHA. Moreover, UV-spectral purities of β-artemether and lumefantrine chromatographic peaks were evaluated using Waters’ peak purity PDA evaluation.

Robustness

A Plackett-Burman experimental design consisting of 12 experiments with two replicates in block was used for the robustness testing (Modde version 8, Umetrics Inc, USA). Three sample solutions (stressed, test sample and reference solutions) were prepared at 100% lc and analysed using different experimental conditions by varying different analytical parameters: flow (0.8, 1.0, and 1.2 ml/min), acetonitrile proportion (50%, 52% and 54%), mobile phase pH (2.8, 3.0 and 3.2), and column temperature (25ºC, 30ºC and 35ºC). β-artemether and lumefantrine contents and different chromatographic characteristics were determined under each condition.
Limit of detection (LoD) and limit of quantitation (LoQ)
Combined standard solutions of β-artemether and lumefantrine were prepared by serial dilutions, ranging from 0.4 to 25.0 µg/ml for β-artemether and 0.2 to 11.5 µg/ml for lumefantrine, and injected onto the chromatographic system. The LoD was defined as the concentration for which a signal-to-noise ratio (S/N) of three was obtained and LoQ was considered to be the concentration at which S/N was 10.

In-silico toxicological predictions
In-silico toxicological study for lumefantrine and its related impurities was reported in previous publication [26]. Exhaustive impurity profiling of β-artemether (including its possible degradants) was also reported [23, 39]. Therefore, to make in-silico toxicological comparative predictions for β-artemether and its identified related impurities, Derek Nexus v2.0 for Windows developed by Lhasa Ltd (Leeds, UK) was used. Derek Nexus® is an expert knowledge-based system, containing descriptions of molecular substructures which have been associated with toxic endpoints (structural alerts), that predicts a probability whether a chemical is toxic in humans, other mammals and bacteria. The program applies structure-activity relationships ((Q)SARs) and expert knowledge rules to derive a reasoned conclusion about the potential toxicity of the query chemical [40-42].

Quality of artemether-based anti-malarial drugs in Ethiopia
Study area
Jimma zone of oromia regional state which is located in the southwest Ethiopia (Figure 2) was selected for this study where almost all of its districts are malarious. Jimma zone harbours Gilgel Gibe hydropower dam, which has already contributed to the aggravation of malaria incidence and prevalence. It is the commercial hub for the southwest part of Ethiopia and thus complex pharmaceutical transactions occur in the region. Currently, there are 7 wholesales (all of them in Jimma city), 71 private drug shops (31 in Jimma city, and 40 in the districts of the zone), 101 public drug shops in health centers (3 in Jimma city, 98 in the districts of Jimma zone), 18 private pharmacies (all of them in Jimma city) and 4 hospital pharmacies in the zone. Informal pharmaceutical markets could also exist even though focused studies in the area are lacking.

Materials
β-artemether and lumefantrine active pharmaceutical ingredients (APIs) were supplied by Dafra Pharma International (B-2300 Turnhout, Belgium) through Drug Quality and Registration (DruQuaR) laboratory of University of Ghent, Belgium. Purified ultra pure water (18.2 MΩ.cm at 25°C) was
obtained by water purification system (Thermofischer Scientific, USA). Ethyl acetate and acetone (Fisher Scientific, UK), methanol (Himedia Labs, India), glacial acetic acid and sulfuric acid (ReAgent Chemical Services, UK) were analytical grade and used as received.

Sample collection
The sampling strategy was defined following the Medicine Quality Assessment Reporting Guidelines (MEDQUARG) as proposed by Newton PN et al., 2009 [43] based on the questions: “Are there poor quality anti-malarial medicines in the formal distribution outlets in Jimma zone? If there are, what is the prevalence?” Moreover, since there is a possible influence of origin and distribution conditions on medicines quality as received by the patient, we included the different formal outlets that are in practice used by patients in Ethiopia. We also looked at the following question: “Is there a difference in quality of medicines (1) among the different levels of medicines outlets? and (2) among the different countries of origin”. Therefore, in function of the questions, sampling units were defined to be the medicines sold from the drug retail outlets of the formal supply chain in the zone, the different levels of the supply chain system in Ethiopia (drug stores including health centers, pharmacies including hospital pharmacies, wholesalers), and country of origin.
Based on the sampling strategy, 74 FDC ACT anti-malarial drug samples were collected between May and June 2013 through random sampling from all the levels of the formal public and private supply chain. All samples were tablet formulations containing artemether 20 mg/lumefantrine 120 mg and collected anonymously by mystery shoppers from local area who were trained before to represent a confirmed \textit{P. falciparum} malaria patient of 25 years of age with a prescription stating 4 tablets twice per day for three consecutive days (full adult dose of 24 tablets).

The mystery shoppers were provided with prescription papers. They were blinded about the purpose of the study and only instructed to collect medicines in their original primary packaging. For the purpose of this study, the relevant information of all collected samples was recorded on a standard form as soon as leaving the drug outlet and entered into a database. The information included the level of the drug outlet, place of collection, name of the active pharmaceutical ingredient, the country of origin, manufacturing company, expiry date, manufacturing date, batch/lot number, and labeled dose (strength) of the active ingredient. Medicines purchased from a specific outlet, labeled with a specific generic name or brand name, strength, number of units per strip/package, batch number, country of origin, manufacturing and expiry dates were considered as one sample. Sampling units were the tablet formulations of artemether/lumefantrine combination products circulating in the zone. Each sample unit consisted of 24 tablets. The samples were stored at ambient temperature (20˚C to 25˚C) until tested, with a storage period of maximally 3 months before testing, and none of samples had expired at the time of testing.

Due to the highest malaria burden reported around Gilgel Gibe dam with incidence rate of 14 cases/1000 child-months at risk [44] and prevalence of 10.5% among children population [45], all the public drug outlets located at 50 km radius surrounding the catchment area of Gilgel Gibe hydropower dam were purposively included in the study. Therefore, all artemether/lumefantrine combination tablet products of different origin and batch were collected from all drug outlets available in these catchment areas and Jimma zone including the city of Jimma.

**Tests for products quality**

World health organization (WHO) checklist was used for visual inspection of packaging and dosage form as a quick means of checking the quality of the samples [46]. The packaging was checked for correct and legible labeling of active ingredients and strength, expiration date, batch number, manufacturer, and country of origin. The tablets were also checked for their physical characteristics (shapes, colour, breaks, cracks and splits).

The laboratory tests were carried out according to individual monographs specified in International Pharmacopoeias (Ph. Int.) [47] and the general monographs described in European Pharmacopoea
CHAPTER IV – QUALITY OF ARTEMETHER-BASED ANTI-MALARIAL DRUGS IN ETHIOPIA

(Ph. Eur) [38, 48] in Jimma University Laboratory of Drug Quality (JuLaDQ), Jimma, Ethiopia. JuLaDQ follows a formal quality system based on ISO and WHO guidelines. All samples were evaluated by determining three clinically relevant critical quality attributes: (i) identification, (ii) mass uniformity, (iii) the amount of the active compounds in the ACT FDC products.

Instrument performance qualification-calibrations as well as system suitability tests (SSTs) were successfully performed for the analytical instruments and HPLC methods, respectively.

**Identification test**

Thin layer chromatography (TLC) was used to determine the presence of β-artemether and lumefantrine in the FPP samples analyzed. Furthermore, chromatographic peak retention time and DAD-UV absorption spectra were used for the purpose of identification through comparison with retention time and DAD absorption spectra of the peak obtained on a reference standard solution of β-artemether and lumefantrine. Customized spectral libraries obtained on the reference standard solutions were established and peak identification for each API in each sample was done by comparing a spectrum from the sample to a spectrum from the established library. The ChemStation software was used to calculate the correlation between the library and the experimental spectra in terms of match factors. A match factor of 1000 (correlation factor = 1) describes identical spectra.

**Uniformity of mass**

Twenty tablets of each of the samples were randomly selected and individually weighed with a calibrated balance (Mettler Toledo, AL204-1C, Switzerland) with an experimentally determined operating range of 95 mg to 200 g and an accuracy of 0.006% [49]. This weight variation test was evaluated against the Ph. Eur. specification [48].

**API content (assay)**

Assay for β-artemether and lumefantrine content of all the samples was performed using individual monographs in Ph. Int.

For assay evaluation of β-artemether and lumefantrine, Agilent 1260 Infinity Series HPLC system (Agilent Technologies, Santa Clara, California, USA) with a C18 column (150 mm x 3.9 mm, 5μ particle size) coupled to diode-array detector (DAD) was used. All HPLC conditions including sample and mobile phase preparations were based on the individual monograph for artemether/lumefantrine tablets described in Ph. Int.

The analytical method was validated according to International Conference on Harmonisation (ICH) Q2(R1) recommendations [50]. The linearity of the method was evaluated around the target label claim concentration (analytical aliquot concentration of 200 µg/ml ranging from 160 to 240 µg/ml for...
β-artemether and 1200 µg/ml ranging from 960 to 1440 µg/ml for lumefantrine. The regression line was assessed by determining the 95% confidence interval (95% CI) of slope and intercept parameters as well as by evaluating the residual plot. Repeatability precision was evaluated by injecting six replicates of 100% test concentration (200 µg/ml for β-artemether and 1200 µg/ml for lumefantrine) and percent relative standard deviation (%RSD) of the measurements was calculated. The accuracy and range of the method was determined by spike experiments at five different concentrations corresponding to 80, 90, 100, 110 and 120% of the nominal analytical concentration for both APIs. System suitability was evaluated by the symmetry factor of both β-artemether and lumefantrine reference standards, calculated using the Ph. Eur. equation $A_s = \frac{w_{0.05}}{2d}$, where, $w_{0.05}$ is width of the peak at one twentieth of the peak height, $d$ is distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one twentieth of the peak height; and injection repeatability by injecting (6 times) the β-artemether and lumefantrine reference standard solution. The Ph.Eur. SST specifications were considered, i.e. $A_s$ maximally 1.5 and %RSD maximally 2 [38].

In addition, the chromatographic profiles obtained with the FPP sample solutions were evaluated for any related impurity above the reporting threshold of 0.05%, recommended for drugs having maximum daily dose <2 g for any impurity according to ICH Q3B guideline [51].

3. RESULTS AND DISCUSSION

HPLC method development and validation

Development

To develop a rapid, simple and stability-indicating isocratic HPLC method, three different fused-core stationary phases (Halo phenyl-hexyl, Halo C18 and Halo RP-Amide) and a mobile phase with different compositions of acetonitrile and 1 mM phosphate buffer with varying pH (3.0, 5.0 and 7.0) were used. Relatively longer run time was obtained with 1 mM phosphate buffer pH 7.0 while pH 5.0 resulted in poor peak shape for lumefantrine. At all conditions, there was no separation between β-artemether and lumefantrine using Halo phenyl-hexyl stationary phase. Using Halo C18 stationary phase column and a mobile phase composed of acetonitrile and 1 mM phosphate buffer pH 3.0, the retention factors obtained for β-artemether and lumefantrine were 11.8 and 3.0, respectively. Under these conditions, in spite of achieving good separation between β-artemether and lumefantrine, the peak shape of lumefantrine was found to be out of pharmacopoeial specifications (Ph Eur. specification $A_s \leq 1.5$) [38] and the total run time was relatively long, i.e., 6 min. Substituting the Halo
C<sub>18</sub> with a Halo RP-Amide stationary phase, different proportions of mobile phase solvents were evaluated. The optimal mobile phase, composed of acetonitrile and 1 mM phosphate buffer pH 3.0 (52:48, V/V), gave an adequate retention factor \( k' \) and lumefantrine peak shape (\( A_2 1.3 \)) that complies with pharmacopoeial specifications within a short period of total run time of 4 min (Figure 3).

Figure 3. Typical chromatogram obtained on solution of β-artemether (Art) (\( t_R \): 3.07 min) and lumefantrine (Lum) (\( t_R \): 1.70 min) in tetrahydrofuran (THF) with their UV spectrum in the infronts.

As indicated in the UV-spectra of Figure 3, β-artemether only shows reasonable UV-absorption at the lower wavelengths of the spectrum (200-230 nm), due to the absence of UV-chromophores in its structure. Thus, its quantification was performed at 210 nm. For lumefantrine with a \( t_R \) of 1.70 min, quantification was performed at 335 nm, the wavelength at which no UV-absorption interference from β-artemether was observed (Table 1).

<table>
<thead>
<tr>
<th>#</th>
<th>β-artemether</th>
<th>DHA</th>
<th>Artemisinin</th>
<th>α-artemether</th>
<th>LEI</th>
<th>lumefantrine</th>
<th>DBK</th>
<th>N-oxide of lumefantrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_R ) (min)</td>
<td>3.069</td>
<td>1.615</td>
<td>1.675</td>
<td>2.142</td>
<td>2.705</td>
<td>1.700</td>
<td>1.256</td>
<td>3.167</td>
</tr>
</tbody>
</table>

Table 1. Retention time \( (t_R) \) for β-artemether and lumefantrine and their related impurities.
It was reported that the desbenzyl keto derivative (DBK) is the major degradation product after acidic degradation of lumefantrine, while the oxidative degradation of lumefantrine was reported to yield N-oxide-lumefantrine [26]. Under the chromatographic conditions, DBK eluted before lumefantrine at retention time ($t_R$) of 1.26 min, while N-oxide-lumefantrine was eluting after lumefantrine at $t_R$ of 3.17 min (Table 1).

β-artemether and all its identified related impurities (α-artemether, DHA, artemisinin and AHA) were eluting at different RT without any interference with the main peak. Previous studies have indicated that AHA-β-artemether is the most critical pair to be separated [39], while other impurities like DKA or furano acetate are sufficiently well separated from β-artemether. Moreover, lumefantrine and its related degradation impurities (DBK and the N-oxide of lumefantrine) were also eluting at different $t_R$. However, while some of β-artemether impurities (DHA, artemisinin and other degradation products from dry heat stress β-artemether) co-elute with lumefantrine, which however does not interfere with its assay due to negligible UV-absorption of these β-artemether impurities at 335 nm, the N-oxide-lumefantrine problematically co-elutes with β-artemether peak, making selective quantification of β-artemether at 210 nm difficult. Therefore, the method quantifies lumefantrine separated from its related impurities at 335 nm (where β-artemether and its related impurities are not absorbing). Since lumefantrine and its related impurities have strong UV absorption at 210 nm and N-oxide-lumefantrine is co-eluting with β-artemether, it is possible to selectively obtain the UV$_{210}$ nm peak area of β-artemether alone by back-calculating the peak area of N-oxide-lumefantrine using the peak area conversion factor from 335 nm to 210 nm (i.e, 1.64) and subtracting the value from the co-eluting peak area at 210 nm.

Therefore, compared to the method described in Ph. Int. [29] which uses conventional HPLC, the developed fused-core method uses simple sample extraction technique and is isocratic, rapid, less costly and stability-indicating.

**Validation**

**Linearity**

Almost all the variation in peak area was explained by the linear concentration (0.9997 for β-artemether and 0.9997 for lumefantrine), indicating the linearity of the method in the assayed range (80 to 120% label claim). The regression analysis data are presented in Table 2.

**Precision**

In the prepared solutions for analysis, 100% label claim (Ic) represents 0.2 mg/ml β-artemether and 1.2 mg/ml lumefantrine solution.
**Intra-day precision:** Mean contents and RSD of β-artemether and lumefantrine in the intra-day precision analysis (n=6) were 99.6% lc with RSD = 1.2% and 99.2% lc with RSD = 0.5%, respectively.

**Inter-day precision:** Mean contents and RSD values of β-artemether and lumefantrine in the inter-day precision analysis (n=3×6) were 99.6% lc with RSD = 1.1% and 99.4% lc with RSD = 0.6%), respectively. For both compounds, the intra-day and inter-day precision % RSD values were lower than 2.0%, revealing precision of the method [52].

| Table 2. Calibration curve for β-artemether and lumefantrine. |
|-----------------|-----------------|-----------------|
| **Regression parameters** | **β-artemether** | **lumefantrine** |
| Regression coeffici | 0.9995 | 0.9996 |
| Slope ± standard error | 99.65±1.33 | 8844.67±97.29 |
| Intercept ± standard error | 589.60±268.83 | -192512.80±117909.37 |
| Concentration range (µg/ml) | 160-240 | 960-1440 |

**Accuracy (recovery test)**

The recovery test was performed by analysing a spiked placebo. β-artemether mean recovery (n=6) was 99.7% (RSD = 0.7%) and lumefantrine mean recovery was 99.7% (RSD = 0.6%), indicating the accuracy of the method.

**Selectivity**

The chromatograms obtained with the stressed lumefantrine API solutions showed degradation impurity peaks separated from the main API peak, and similar findings were observed for the stressed β-artemether solutions. Acid-stressed lumefantrine resulted in DBK eluting before lumefantrine at t_R of 1.26 min, while oxidative stress resulted in N-oxide-lumefantrine eluting after lumefantrine at t_R of 3.17 min. β-artemether and all its identified related impurities (α-artemether, DHA, artemisinin and AHA) were eluting at different t_R without any interference with the β-artemether peak. However, at 210 nm, N-oxide-lumefantrine was co-eluting with β-artemether and some degradants of β-artemether were co-eluting with lumefantrine. There is no interference from β-artemether and its impurities for the estimation of lumefantrine and its related impurities at 335 nm. Therefore, for the quantification of the two APIs, the method uses two wavelengths, 210 nm for β-artemether and 335 nm for lumefantrine.

The peak purity indices for both β-artemether and lumefantrine in different marketed FDC antimalarial drug sample solutions determined with PDA detector under optimized chromatographic conditions indicated that the purity angle for both APIs was less than the purity threshold, revealing no significant excipient interference.
**Robustness**

A Plackett-Burman design was used to test the robustness of the method. Plackett-Burman design is a two level fractional factorial design where main effects are heavily confounded with two factor interactions. It is selected for robustness evaluation since it combines less experimentation with maximal information acquisition in the most efficient way.

Four factors, with deliberate small deviations from the method settings, were considered: percentage V/V of acetonitrile in mobile phase (from 50 to 54%), flow (from 0.8 to 1.2 ml/min), pH (from 2.8 to 3.2) and column temperature (from 25 to 35°C).

Mobile phase pH significantly affects the peak shape of lumefantrine while it did not reveal prominent influence on that of β-artemether. Thus, lumefantrine peak symmetry was selected as a critical quality parameter for the robustness test. The final method provided lumefantrine peak shape (A_s 1.3) that complies with pharmacopoeial specifications. Moreover, even the deliberate method variations provided better lumefantrine peak shapes (A_s 1.4 and 1.8) than the much tailed lumefantrine peak shape (A_s 2.1) reported in the literature [26]. In the stressed sample solutions, there was no difference in selectivity between the results of the method setting and the deliberate variations of both β-artemether and lumefantrine APIs and their respective degradation products.

Typical contour plots for different chromatographic parameters as a function of operational variables levels is presented in Figure 4. Figure 4 (a, b and c) is the visual representation of sensitivity, i.e. how quantitatively acetonitrile proportion (% ACN), flow rate and temperature influence the retention factor (k’). Moreover, it is revealed in Figure 4 that small deviations from the method setting introduced in the four parameters do not affect A_s and k’-specifications set in Ph. Eur. [38]. The observed effects for peak symmetry (A_s) of lumefantrine and k’ for β-artemether and lumefantrine are presented in Figure 5. Flow rate and %ACN have more pronounced effect on k’ of both compounds while lumefantrine peak shape was more affected by % ACN.

The mean content of β-artemether and lumefantrine was found to be 100.9 % lc ± 1.0 (RSD 1.0 %) and 99.7 % lc ± 0.4 (RSD 0.4 %), respectively. Therefore, the deliberate changes from the method settings in chromatographic conditions (% V/V of acetonitrile in mobile phase (from 50 to 54 %), flow (from 0.8 to 1.2 ml/min), pH (from 2.8 to 3.2) and column temperature (from 25 to 35°C)) have little impact on the assay of β-artemether and lumefantrine containing anti-malarial FDC products indicating the robustness of the method.
Figure 4. Contour plots: (a) Acetonitrile (ACN) (% V/V) vs flow (ml/min) for retention factor ($k'$) of β-artemether, (b) ACN (% V/V) vs flow (ml/min) for $k'$ of lumefantrine, (c) Temperature (˚C) vs flow (ml/min) for $k'$ of β-artemether, (d) ACN (% V/V) vs flow (ml/min) for $A_s$ of lumefantrine. For (a), (b) and (d) mobile phase: pH 3, Column temperature: 30 °C and for (c) % ACN 52, mobile phase: pH 3.

Limit of detection (LoD) and limit of quantitation (LoQ)

According to the determined signal-to-noise ratio, the LoD and LoQ for β-artemether were calculated to be 3.4 µg/ml and 10.0 µg/ml, respectively. For lumefantrine, LoD was 0.1 µg/ml and its LoQ was 0.4 µg/ml. As the purpose of this developed method is to quantitatively determine both β-artemether and lumefantrine simultaneously in FDC anti-malarial products where the compounds exist in the mass ratio β-artemether: lumefantrine of 1:6, the LoD and LoQ values obtained for β-artemether should be considered as the overall detection and quantification limits, while for lumefantrine, the risk of overloading the HPLC system is to be considered. Both opposing aspects are solved with the proposed method.
Analysis of marketed FDC products

The results of real sample analysis are presented in Table 3. All the analysed batches presented β-artemether and lumefantrine contents complying with the 95-105% \text{lc} specifications. The β-artemether content in the tablet samples varied from 98.2% to 103.2% while lumefantrine content varied from 97.9% to 101.5%. In Co-Artesiane powder for oral suspension FDC product, β-artemether content was in the range of 99.7% to 101.1% while that of lumefantrine was ranging from 100.8% to 102.0%.

\textit{In-silico} toxicological predictions of β-artemether and its related impurities

\textit{In-silico} toxicity profile of lumefantrine and its related impurities was reported in previous publication [26]. In this study, mutagenicity, chromosome abrasion, genotoxicity, skin irritation, hepatotoxicity and nephrotoxicity endpoints for β-artemether, as well as for its related observed and already described impurities, have been investigated using Derek Nexus\textsuperscript{®} and the result is presented in Figure 6. The toxicity profile of β-artemether and all its identified related degradants and synthetic impurities is defined by several general toxicity alerts. DHA, α-artemether and β-artemether were found to have toxicity endpoints for mutagenicity, chromosomal abrasion, genotoxicity, skin
irritation, hepatogenicity and nephrotoxicity. β-artemether and all its identified related impurities, except desoxyartemisinin which has structural alert for hepatotoxicity, have substructures for skin irritation. Derek Nexus® did not trigger mutagenicity, chromosomal abrasion and genotoxicity for artemisinin, 9-epi artemisinin, artemisitene, desoxyartemisinin and AHA.

**Table 3.** Contents of β-artemether and lumefantrine in fixed dose combination (FDC) products (n=6).

<table>
<thead>
<tr>
<th>FDC samples</th>
<th>Batch/Lot No.</th>
<th>Content (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>β-artemether</td>
</tr>
<tr>
<td>Artemine® tablets</td>
<td>A</td>
<td>102.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>102.0 ± 1.2</td>
</tr>
<tr>
<td>Coartem® tablets</td>
<td>A</td>
<td>98.5 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>99.5 ± 0.7</td>
</tr>
<tr>
<td>Co-Artesiane powder for oral suspension</td>
<td>A</td>
<td>103.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>102.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>103.7 ± 1.1</td>
</tr>
</tbody>
</table>

SD = standard deviation

**Quality survey**

A total of 74 FDC ACT anti-malarial drug samples containing β-artemether (20 mg) and lumefantrine (120 mg) were collected between May and June 2013 from malarious districts of Jimma zone. The samples had been collected from 27 premises (wholesales, hospital pharmacies and health center drug stores). Of these, 34 samples were Coartem®, USA; whereas 6 samples were Artefan® and 32 samples were generic products of artemether/lumefantrine from India. One sample was Coartem® which was manufactured in China whereas 1 Artemine® sample was Ethiopian origin. The origin (place of manufacturing) of samples was domestic and foreign (China, India, and USA).

**Visual inspection for packaging and dosage form**

The visual inspection of the dosage forms and packaging showed 100% compliance of the samples with the requirements set by WHO [53]. Labeling information regarding dosage form, brand name, active ingredient/strength, batch number, manufacture and expiry dates were provided. Tablets did not present with non-uniform coloration or signs of breakage.

Evaluation of quality of medicines starts with checking the packaging/labeling and dosage form of the medicine samples [54]. Thus, visual inspection for signs of poor quality such as improper packaging, labeling, description of dosage, and product source/origin was carried out using WHO checklist [46].
## CHAPTER IV – QUALITY OF ARTEMETHER-BASED ANTI-MALARIAL DRUGS IN ETHIOPIA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Manager-nic</th>
<th>Chromat</th>
<th>Genotoxic</th>
<th>Skin</th>
<th>Irritation</th>
<th>Hepatotoxic</th>
<th>Neurotoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-artemether</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3R,5aS,6R,8aS,10R,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin-10(3H)-one</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemisinin</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3R,5aS,6R,8aS,9R,10S,12R,12aR)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin-10(3H)-one</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-artemether</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3R,5aS,6R,8aS,9R,10S,12R,12aR)-Decahydro-10-methoxy-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-epi artemisin</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>1R,5R,12S)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo-hexadecan-10-one</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artemisitene</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3,12-Epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin-10(3H)-one,octahydro-3,6-dimethyl-9-methylene- (3R,5aS,6R,8aS,12S,12aR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desoxyartemisin</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>[(1R,9R)-1,5,9-trimethyl-11,14,15-tetraoxatetracyclo-hexadecan-10-one</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dihydrartemisinin (DHA)</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3R,5aS,6R,8aS,10S,12R,12aR)-Decahydro-10-hydroxy-3,6,9-trimethyl-3,12-epoxy-12H-pyran[4,3-j]-1,2-benzodioxepin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diketo aldehyde (DKA)</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>2-[4-methyl-2-oxo-3-(3-oxobutyl)cyclohexyl]propanal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furano acetate</td>
<td><img src="image9.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>(3aS,4R,6aS,7R,8S,10R,10aR)-8-methoxy-4,7-dimethoxycyclohexydro-2H-furo[3,2-j][2]benzopyran-10-yl acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9,10-anhydroartemisinin (AIA)</td>
<td><img src="image10.png" alt="Structure" /></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><a href="9,10-anhydroartemisinin">9,10-anhydro-10-deoxyartemisinin</a></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Presence of structural alerts.

**Figure 6.** *In-silico* toxicity profile of β-artemether and its impurities.

The visual inspection did not find obvious falsified packaging and/or mislabeling of the surveyed medicines, which goes in line with previous reports [55, 56]. This can be explained by the fact that
the investigated ACT anti-malarial samples were those supplied to government owned public facilities through donation and patients can access free of charge. Therefore, this zero percent failure rate with regard to packaging and labeling does not necessarily proof the efficacy of the overall regulatory system in Ethiopia, as for example poor quality albendazole and mebendazole products were previously found [56].

Weak pharmaceutical regulatory system in general and weak regulation of medicines packaging and labeling in particular has more pronounced effect on the spread of poor quality medicines [57]. Since packaging preserves the stability and quality of medicinal products as well as protects them against all forms of spoilage and tampering, and since the information on the labels, packaging and leaflets provide the basis for appropriate use, WHO recommends that all medicinal products need to be packaged in containers that conform to prescribed standards with careful labeling [58]. Different countries have different regulatory frameworks and guidelines to control packaging and labeling of medicines with the same primary objective, i.e. the unambiguous identification of the medicines and the clear conditions for their efficacious and safe use. According to food, medicine and health care administration and control proclamation 661/2009 of Ethiopia, any producer, importer, distributor, retailer or health institution of medicine shall not supply it to the market or distribute it otherwise unless it is duly packed and labeled. Similarly, regulation (EC) No 726/2004 and directive 2001/83/EC of European Union (EU) have provisions on the text of the label and package of medicinal products placed on the EU market. The US Food and Drug Administration (FDA) regulates packaging and labeling of medicines using Code of Federal Regulations Title 21 Part 211 (21CFR211) Subpart G - Packaging and Labeling Control. Overview of the regulatory aspect of pharmaceutical packaging in Ethiopia in comparison to those of WHO, EU and USA is described in Table 4. Even though Ethiopia does have legal framework for pharmaceutical packaging and labeling control, there is no consolidated regulatory guideline and/or inspection checklist contrasting WHO, EU and USA. For the regulatory control of packaging (including packaging materials) and labeling, Ethiopia uses its GMP guideline for pharmaceutical products which contains specifications for packaging materials, packaging and labeling operations and instructions [59].

Such packaging and labeling control through careful visual inspection must be followed by chemical analysis, most often using HPLC, which is considered as the gold standard analytical method in drug analysis [54]. Therefore, quality evaluation using identification, mass uniformity and assay as critical quality attributes was conducted based on the conventional viewpoint, i.e. the dichotomous acceptance/reject decision based on the pharmacopoeial specifications, which contrasts the desirability approach [56]. This dichotomous perspective was based on the acceptance criteria set in the individual monographs of Ph. Int. [47] and the general monographs of Ph. Eur. [38].
## Table 4. An overview of regulatory aspects of pharmaceutical packaging in Ethiopia in comparison with WHO, EU and USA.

<table>
<thead>
<tr>
<th></th>
<th>Features</th>
<th>Ethiopia</th>
<th>WHO</th>
<th>EU</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Guidelines for pharmaceutical packaging</td>
<td>No separate guideline but part of the GMP guideline for pharmaceutical products</td>
<td>Guideline on packaging for pharmaceutical products</td>
<td>Guideline on plastic immediate packaging materials CPMP/QWP/4359/0 3, EMEA/CVMP/205/04</td>
<td>FDA guidance for industry “Container Closure Systems for Packaging Human Drugs and Biologics”</td>
</tr>
<tr>
<td>3</td>
<td>Standard monographs for pharmaceutical packaging requirements</td>
<td>No Pharmacopoeia, but uses monographs in other Pharmacopoeias: USP, Ph. Eur, and Ph. Int.</td>
<td>International Pharmacopoeia (Ph.Int.)</td>
<td>European Pharmacopoeia (Ph.Eur.)</td>
<td>United States Pharmacopoeia (USP)</td>
</tr>
<tr>
<td>4</td>
<td>Presence of checklist for visual inspection of packaging and dosage form</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Regulatory requirements for pharmaceutical packaging</td>
<td>Protection of the drug product (chemical and physical stability, protection from mechanical damage and maintaining product integrity), provision of all the necessary information about the medicine (identification including strength, batch, or expiration date and proper physical characteristics (no moisture, dirty marks, abrasion erosion, cracks, or any other adulterations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Identification tests
All samples had the intended active ingredient as demonstrated by the positive identification tests.

Uniformity of mass
The results of uniformity of mass for ABZ tablets of each brand are presented in Table 5. The results revealed that all analyzed samples complied with pharmacopoeial specification limit [48].

Table 5. Mass uniformity test results (% deviation) distribution among products of FDC ACT.

<table>
<thead>
<tr>
<th>#</th>
<th>Brand/generic drug product (n)</th>
<th>%Deviation Minimum</th>
<th>%Deviation Maximum</th>
<th>Mean %Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coartem (35)</td>
<td>0.75</td>
<td>7.11</td>
<td>1.61</td>
</tr>
<tr>
<td>2</td>
<td>Generic (32)</td>
<td>0.80</td>
<td>2.64</td>
<td>1.78</td>
</tr>
<tr>
<td>3</td>
<td>Artefan (6)</td>
<td>0.99</td>
<td>4.50</td>
<td>2.55</td>
</tr>
<tr>
<td>4</td>
<td>Artemine (1)</td>
<td>1.34</td>
<td>1.34</td>
<td>1.34</td>
</tr>
</tbody>
</table>

API content (Assay)
The validation and SST results of the HPLC method revealed that the test method is fit for the intended purpose. The method was linear over the concentration range of 160 to 240 µg/ml for β-artemether ($r^2 = 0.9998$) and 960 to 1440 µg/ml for lumefantrine ($r^2 = 0.9999$). The regression analysis results for method linearity are presented in Table 6. Percentage relative standard deviation for repeatability (%RSD= 0.85 for β-artemether and %RSD = 0.76 for lumefantrine) of the method were within the specification limit (%RSD ≤2). The results of percent recovery (mean % ± %RSD = 99.60 ± 0.76% for β-artemether and mean % ± %RSD = 99.92 ± 0.58 % for lumefantrine) were within acceptable range. The symmetry factor of β-artemether was found to be 1.0 while that of lumefantrine was 0.9, which reveals compliance with the Ph.Eur. SST specifications [38].

Table 6. The HPLC method linearity for assay of β-artemether and lumefantrine.

<table>
<thead>
<tr>
<th>Regression parameters</th>
<th>β-artemether</th>
<th>lumefantrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficient, $r^2$</td>
<td>0.9998</td>
<td>0.9999</td>
</tr>
<tr>
<td>Slope ± standard error</td>
<td>$1.15 ± 0.01$</td>
<td>$80.65 ± 0.15$</td>
</tr>
<tr>
<td>Intercept ± standard error</td>
<td>$10.34 ± 0.71$</td>
<td>$8.12 ± 18.06$</td>
</tr>
<tr>
<td>Concentration range (µg/ml)</td>
<td>160-240</td>
<td>960-1440</td>
</tr>
</tbody>
</table>
With regard to both β-artemether and lumefantrine content in the tablet dosage forms analyzed, all the samples analyzed comply with the acceptance specification set in Ph. Int. [47], *i.e.* 90 -110 % label claim (% lc), except one generic product failed with overage of 111.9% lc for lumefantrine. Therefore, in all the samples analyzed, β-artemether content ranges from 89.8 to 108.8% (mean 99.1%, SD: 3.9%), while that of lumefantrine content was from 90.0 to 111.9% (mean 98.2%, SD: 3.8%).

A box plot of assay for both β-artemether and lumefantrine by product type (brand and/or generic) and country of origin is indicated in Figure 7 and assay test results by product brand and/or generic is presented in Table 7.

### Table 7. Assay test results for both β-artemether and lumefantrine by product brand and/generic type (in percent label claim).

<table>
<thead>
<tr>
<th>#</th>
<th>Brand/generic drug product (n)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coartem® (35)</td>
<td>β-artemether</td>
<td>90.9</td>
<td>103.6</td>
<td>98.9</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumefantrine</td>
<td>90.0</td>
<td>104.1</td>
<td>97.4</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>Generic (32)</td>
<td>β-artemether</td>
<td>91.5</td>
<td>106.7</td>
<td>99.2</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumefantrine</td>
<td>93.6</td>
<td>111.9</td>
<td>98.8</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>Artefan® (6)</td>
<td>β-artemether</td>
<td>89.8</td>
<td>103.4</td>
<td>98.2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumefantrine</td>
<td>91.2</td>
<td>104.2</td>
<td>98.7</td>
<td>4.7</td>
</tr>
<tr>
<td>4</td>
<td>Artemine® (1)</td>
<td>β-artemether</td>
<td>108.8</td>
<td>108.8</td>
<td>108.8</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lumefantrine</td>
<td>108.0</td>
<td>108.0</td>
<td>108.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

SD: standard deviation; NA: not applicable

With regard to β-artemether and lumefantrine related impurities, the chromatographic peaks obtained on solutions of the tablet samples were evaluated for any impurity according to ICH Q3B guideline and all samples did not reveal any related impurity above the reporting threshold of 0.05% recommended for drugs having maximum daily dose < 2 g.

In this quality survey study, the semantics ‘poor quality’ was used since it represents both substandard and counterfeit medicines [34] and there were no obvious features which suggest that the investigated samples were counterfeit, falsified and/or unregistered; although the study did not explicitly search for counterfeits, falsified and/or unregistered medicines.

All the analyzed drug products complied with the stated pharmacopeial acceptance criteria for the three quality attributes: identification, mass uniformity and assay. The zero failure rate observed in this survey was found to be similar with a study conducted across Ethiopia [55], despite the reports on poor quality anti-malarials, both non-artemisinin and artemisinin containing, in Africa [55, 60]. However, this cannot be fully explained by the efficiency of the overall regulatory system, as it can be
revealed (1) in the WHO survey that 41% of the collected samples were not registered by the national medicine regulatory authority [55]; (2) a national quality survey conducted in Ethiopia on three other products (albendazole, mebendazole and tinidazole) indicated the high prevalence of poor quality products (45.3%) for the three products [56]. This might be due to the fact that the surveyed medicines in this study were collected from strictly regulated public facilities, while the previous reports included both licensed and unlicensed medicines retail outlets, indicating that the risk of poor quality medicines is greatest in the unregulated pharmaceutical sectors.

Figure 7. Box plot for assay of artemether and lumefantrine by product brand-generic and by country of origin.

Although the sample size was too small to generalize, there was a significant difference in the assay values for both β-artemether and lumefantrine in the analyzed fixed dose combination products (FPPs) between countries of origin (P < 0.05) but there was no significant association for place of collection and brands and/or generic products (P > 0.05).
Contrasting the public health facilities explored in this study, where those medicines are given without payment to the patients, in the private sector, significant price differences between products of different origin are observed. While the availability of artemether/lumefantrine FDC tablet products in the private sector is low, where available, the prices for a full adult dose of 24 tablets are immensely high, even for the lowest-priced generic (ETB 88 = 4.4 USD) and between ETB 96 (4.8 USD) and ETB 199 (10.0 USD) for the originator brand (Coartem®) (Exchange rate: 1 USD = 20 ETB). This significantly high price makes the medicines unaffordable for the majority of malaria patients at the private sector [61]. Therefore, as part of improving access to malaria treatment, ACT tablets are dispensed to patients free of charge at public health facilities. This effort has significantly and positively contributed to the malaria prevention and control strategies set by the country [62]. Despite those encouraging progresses, evidence reveals frequent stock depletions and shortages of such drugs at the formal, public health facilities [63]. Consequently, patients often must visit the private sector for an alternative source of ACTs. Therefore, with extremely limited stocks of ACTs by private facilities, expanding the involvement of the private sector toward the availability and rational use of ACTs has been recommended [61], which will require an extended strict regulatory control system implemented to assure the quality of these medicines through the private channel as well.

4. CONCLUSIONS

A stability-indicating HPLC method for simultaneous assay of β-artemether and lumefantrine fixed dose combination anti-malarial products was developed, using a fused-core reversed-phase amide stationary phase combined with an isocratic acetonitrile sodium phosphate mobile phase [Acetonitrile/1 mM phosphate buffer pH 3.0 (52:48, v/v)]. It is a rapid (four minutes total run time), precise and accurate method that can be utilized to quantify these anti-malarials in the presence of their related degradation products or impurities produced during inadequate transportation and storage. This method can be applied in the routine regulatory quality control of β-artemether and lumefantrine containing FDC drug products. The in-silico toxicological investigation using Derek Nexus® indicated overall a toxicity risk for β-artemether-related impurities comparable to that of the API β-artemether itself.

From visual inspection, the quality survey study revealed that there were no falsified packages and labels for the samples obtained from formal outlets. All the sampled ACT products did contain the stated active ingredient and all (except one sample with 111% lc for lumefantrine) comply with the acceptance criteria set in Ph. Int. for assay (90-110 % lc) and Ph. Eur. for mass uniformity. Even
though this is good for the malaria control program of the country, it does not necessarily imply the
efficiency of the overall national regulatory system of Ethiopia, including the private sector as well.
Therefore, regulatory and policy strategies including nationwide surveys of quality of medicines for
the containment of poor quality anti-malarials should always be active and implemented.

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CHAPTER V

QUALITY OF MEDICINES FOR THE TREATMENT OF SOIL TRANSMITTED HELMINTHS AND GIARDIA IN ETHIOPIA: A NATIONWIDE SURVEY

“If you cannot measure it, you cannot improve it.”

Lord Kelvin
(*1824 - †1907, Irish mathematical physicist)

Parts of this chapter were published:

ABSTRACT

Access to medicines of good quality improve the chances of successful treatment for individual patients and promote better outcomes for public health in general. At present, the prevailing strategy for improving access to medicines for neglected tropical diseases (NTDs) is drug donation programs. However, the presence of poor quality medicines in the market is a global threat on public health, especially in developing countries by critically risking efforts of treatment and control of diseases in general and the NTDs in particular. Conventionally, medicine quality has been ignored in NTDs, though scattered reports show that serious problems exist. Therefore, we assessed the quality of two commonly used anthelminthic drugs (MEB and ALB) and one antiprotozoal drug (TNZ) in Ethiopia. The analytical results were converted into conclusions using two systems: the traditional dichotomous pharmacopoeial specification-compliance based approach and the risk-based Taguchi quantitative desirability approach. Overall, the results showed high prevalence of poor quality of the three medicines, mainly determined by the country of origin. We conclude that risk-based regulatory quality control procedures should be based on identification of the most critical quality attribute and apply desirability functions to quantify and classify the quality of medicines.
CHAPTER V

QUALITY OF MEDICINES USED FOR THE TREATMENT OF SOIL TRANSMITTED HELMINTHS AND GIARDIA IN ETHIOPIA: A NATIONWIDE SURVEY

Main focus in this chapter:
 To assess the pharmacopoeial quality of three medicines (mebendazole, albendazole and tinidazole) circulating in Ethiopia.
 To assess the criticality of quality attributes and apply risk-based desirability function approach to quality evaluation of medicines.

1. INTRODUCTION

Intestinal parasites are a diverse group of organisms that include single-celled protozoans and multicellular intestinal helminths that affect the gastro-intestinal tract of humans and other animals [1]. Soil-transmitted helminthiasis is caused primarily by four species of nematodes, i.e. *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Ancylostoma duodenale* and *Necator americanus* (hookworms) that parasitize human gastrointestinal tract [2]. These major human soil-transmitted helminths (STH) have significant impact on human health in many parts of the world, particularly in developing countries [3]. If not treated early and efficacious, they may lead to malnutrition, chronic diarrhea, anemia, and other public health problems that can impair physical and intellectual development in children [4-6].

Currently, four drugs are recommended by the World Health Organization (WHO) for STH: MEB, ALB, levamisole and pyrantel pamoate [7, 8]. MEB and ALB are increasingly deployed in mass drug
administration programs [8] which require a single drug administration to all subjects without prior
diagnosis or checking for contra-indications. For this reason, the two benzimidazole 2-carbamates
MEB and ALB (Figure 1) are preferred over levamisole and pyrantel pamoate, which require weight-
based dosing and which are also intrinsically less potent.

![Figure 1. Chemical structure of albendazole (ALB), mebendazole (MEB) and tinidazole (TNZ).](image)

Literature reports indicate that TNZ, a 5-nitroimidazole compound (Figure 1), also has some
anthelmintic efficacy [9], although it is therapeutically mainly used against protozoan infections and
infections caused by anaerobic bacteria in humans. As such TNZ is often used by the same patients
treated with STH drugs [10, 11].

Effective treatment and prevention strategies for these neglected tropical diseases can be delivered
cheaply, but reports of treatment failure are frequent in developing countries most likely because of
poor quality medicines, which includes spurious/falsely labeled/falsified/counterfeit (SFFC)
medicines, chemical and/or physicochemical instability, inappropriate storage and transport, and
poor quality control during manufacturing and importing medicines [12]. SFFC medicines are
medicines that are deliberately and fraudulently mislabeled with respect to identity and/or source
and include products with the correct ingredients or with wrong ingredients, without active
ingredients, with insufficient or too much active ingredient, or with fake packaging [13]. Substandard
medicines, i.e. not having the appropriate quality (which is expected to be equivalent to the
regulatory quality), may be SFFC but also approved medicines. In a quality survey in Nigeria, 48% of
the samples of different categories of medicines were found to be outside the British Pharmacopoeia
(BP) limits for active pharmaceutical ingredient (API) assay. Some medicines were even lacking the
active ingredient [14]. The use of substandard medicines may result in therapeutic failure, resistance
development, and occurrence of serious adverse events or even death due to excessive dose or the
presence of toxic impurities [15-17]. A study conducted in sub-Saharan Africa in 2010 on the quality
of selected anti-malarial medicines reported 64% overall quality failure in Nigeria, from which one
artemisinin-based anti-malarial drug sample did not contain any of artemether API [18].
The presence of substandard and SFFC medicines not only poses threats to the individual users in
terms of the health and side effects experienced, but also to the public and government in terms of
trade relations and economic implications [19]. Hence, like many other public health problems, the issue of the presence of these substandard and SFFC medicines for public consumption should receive careful attention in developing countries [16].

Finished pharmaceutical products (FPPs) are tested for quality by assessing whether they meet pharmacopoeial or any other approved specifications. If not, they are discarded as non-conforming. This is a dichotomous decision without differentiation of the seriousness of failure and/or importance of quality attributes towards clinical use for the patient [20]. The evaluation of quality of any product poses thus a common problem due to a multiplicity of measures which must be balanced one against the other. Even when the quality attributes are precisely measurable, a serious challenge exists in combining the individual measurements into one index representing the total quality [21]. Such balance problems can be solved by using a Derringer’s desirability function [22].

In general, this study was carried out to assess the pharmacopoeial quality of three medicines (MEB, ALB and TNZ) circulating in Ethiopia. The quality in terms of quality attributes like assay/content, dosage uniformity, dissolution, disintegration and friability was evaluated. The criticality of the quality attributes was assessed using FMEA risk-based analysis and Derringer’s desirability function was applied to obtain one global quality index for each sample investigated.

2. MATERIALS AND METHODS

Materials

MEB USP working standard [Cadila Pharmaceuticals (Ethiopia)], ALB reference standard [Greenfield Pharmaceuticals (China)] and TNZ reference standard [Greenfield Pharmaceuticals (China)] were kindly donated from Food, Medicine and Health-care Administration and Control Authority (FMHACA) of Ethiopia and used as received. Purified ultra pure water was obtained by water purification system (Thermofischer Scientific, USA, 18.2 MΩ.cm at 25°C). All other chemicals used in this study were analytical grade and used as received.

Sample Collection

The sampling strategy was defined following the Medicine Quality Assessment Reporting Guidelines (MEDQUARG) as proposed by Newton PN et al., 2009 [23] based on the questions: “Are there medicines of poor quality in the formal distribution outlets in Ethiopia? If there are, what is the prevalence of these poor quality medicines?” Moreover, since there is a possible influence of origin and distribution conditions on medicines quality as received by the patient, we included the different
formal outlets that are in practice used by patients in Ethiopia. So, we also looked at the following question: “Is there a difference in quality of medicines (1) among the different levels of medicines outlets? (2) across different geographic areas of the country? (3) among the two national economies: government and privately owned medicines outlets and (4) among the different countries of origin”. Therefore, in function of the questions, sampling units were defined to be the medicines sold from the drug retail outlets of the formal supply chain in the country, the different levels of the legal (registered) supply chain system in Ethiopia (drug stores incl. health centers, pharmacies incl. hospital pharmacies, wholesalers), the geographic areas, government/privately owned medicines outlets and country of origin. According to the legal pharmaceutical supply system in Ethiopia, the distinction between pharmacy and drug store is based on the dimension of the store and the degree of specialization of the professionals employed. Pharmacies can handle broader scope of medicines than drug store/shop, and are run by a professional with a pharmacist degree. Drug stores/shops are run by a pharmacy technician.

Based on the sampling strategy, 106 drug samples were collected between January and March 2012 through multilevel stratified random sampling from all the levels of the supply chain system of the country (n = 3) covering all types of government and privately owned drug outlets (n = 2). All available drug samples of the three study medicines were collected from each of the selected drug outlet. Through proportional allocation to each stratum of the supply chain, 59.4% (n = 63) of the drug samples were collected from drug stores; 36.8% (n = 39) were from pharmacies while the remaining 3.8% (n = 4) samples were from wholesalers. 17.9% (n = 7) of pharmacy collected drug samples were obtained from hospitals, while four of the drug samples collected from drug stores was from health centers. Depending on the geographic locations and drug markets, the samples were collected from 7 major cities of the country: Addis Ababa, Hawasa (and its region including Arbaminch and Shashemene), Jimma, Assosa (and its region including Nekemte), Adama, Mekele and Bahirdar; which represent all four directions starting from Addis Ababa, the major central commercial center. All samples were tablet formulations and purchased anonymously by mystery shoppers from local area who were trained before. The mystery shoppers stated, if needed, that they were a travelling five member family and the family head, a man of 35 years old, abruptly caught a stomach ache (‘kurtet’ in Amharic) due to worm infestations and requested the dispenser at the medicine outlet for some mebendazole (for ‘kurtet’) and albendazole tablets (for ascariasis) as he used both medicines from his past experiences. At the same time, the family’s 18 years old son was suffering from diarrhea and thus requested the dispenser for any medicines which could be given for him describing that he was taking tinidazole tablets two months ago for similar symptoms. Since the travelling family was in a worry of coming up with shortage of the medicines while travelling they requested a sufficient quantity of tablets of the medicines.
The mystery shoppers were blinded about the purpose of the study and only instructed to purchase medicines in their original primary packaging as supplied by the manufacturer. For the purpose of this study, the relevant information of all collected samples was recorded on a standard form as soon as leaving the drug outlet and entered into database. The information included the level of the drug outlet, place/city of collection, name of the active pharmaceutical ingredient, the country of origin, manufacturing company, expiry date, manufacturing date, batch/lot number, and labeled dose (strength) of the active ingredient. Medicines purchased from a specific outlet, labeled with a specific generic name or brand name, strength, number of units per strip/package, batch number, country of origin, manufacturing and expiry dates were considered as one sample. Since the mystery shoppers stated that they were a travelling five member family, they were able to buy enough units per sample. For MEB, 50 tablets per sample were purchased while for ALB and TNZ, a sample contained 100 tablets. The samples were stored at ambient temperature (20˚C to 25˚C) until tested, with a storage period of maximally 3 months before testing, and none of samples had expired at the time of testing.

**Test Methods for Product Quality**

The quality control laboratory tests were performed in Jimma University Laboratory of Drug Quality (JuLaDQ), Jimma, Ethiopia. JuLaDQ follows a quality system based on WHO and ISO guidelines. The laboratory tests were carried out according to the general and individual monographs specified in different Pharmacopoeias, as indicated in Table 1. Instrument performance and system suitability tests were successfully performed for the analytical instruments and HPLC methods, respectively.

For any drug product, identification of the active pharmaceutical ingredient (API) is a critical quality attribute. The three drugs (ALB, MEB and TNZ) belong to biopharmaceutical classification system (BCS) class II, with low aqueous solubility and high permeability [24, 25]. Moreover, disintegration is an integral part of and/or pre-requisite for dissolution of immediate release dosage forms [26]. Therefore, quality attributes based upon which the products were evaluated were defined to be identification, assay/content, dissolution, dosage uniformity, disintegration and friability tests. Quality failure was defined as a sample failing any single test of the aforementioned tests for which it was evaluated.
Table 1. Quality attributes and corresponding pharmacopoeial specifications for ALB, MEB and TNZ tablet products.

<table>
<thead>
<tr>
<th>#</th>
<th>Attributes</th>
<th>Product</th>
<th>Specifications</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALB, MEB, TNZ</td>
<td>USP</td>
<td>BP</td>
</tr>
<tr>
<td>1</td>
<td>Assay (% lc)</td>
<td></td>
<td>90.0-110.0</td>
<td>NA, NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No individual monographs in BP and Ph Eur</td>
</tr>
<tr>
<td>2</td>
<td>Disintegration time (min)</td>
<td>ALB, TNZ</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The specification is based on general monographs</td>
</tr>
<tr>
<td>3</td>
<td>Dissolution (% dissolved within specified time, min)</td>
<td>ALB</td>
<td>Not &lt;80% in 30 min</td>
<td>Not &lt; 70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The specifications are based on individual monographs for USP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNZ</td>
<td>Not &lt;75% in 120 min</td>
<td>Not &lt; 70</td>
</tr>
<tr>
<td>4</td>
<td>Dosage uniformity (% lc)</td>
<td>ALB, MEB, TNZ</td>
<td>85.0-115.0</td>
<td>85.0-115.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The specification is based on general monographs</td>
</tr>
<tr>
<td>5</td>
<td>Friability (% loss of mass)</td>
<td>ALB, MEB, TNZ</td>
<td>&lt;1</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

NA: Not applicable. Q: the specified amount of dissolved active substance expressed as %lc.

Identification test
Thin layer chromatography (TLC) was used to determine the presence of ALB and MEB, whereas TNZ was identified using UV-spectrophotometry (CECIL CE7200, England) based on the measurement of specific absorbance at 310 nm according to USP 2007.

Assay
Assay for all the three FPPs was performed using individual monographs in USP 2007. For assay evaluation of MEB and ALB, reversed phase (RP) HPLC system (KONIK HPLC 550A, Model 600, Spain) with LC software coupled to UV-VIS detector was used. The separation technique was carried out using C18, 4.6 mm × 150 mm analytical column that contains 5-µm packing. The HPLC conditions were detection wavelength of 254 nm (Konik 600 UV-VIS detector), isocratic flow rate of 1 ml/min and mobile phase consisting of methanol and 10 mM monobasic ammonium phosphate buffer of pH 5.5 (60:40). Before conducting the assays, the HPLC method used was validated for linearity, precision and accuracy.

ALB sample solutions were prepared from 20 tablets which were weighed and finely powdered. A portion of the powder equivalent to 100 mg of ALB was transferred to a 50.0 ml volumetric flask. Then, 5.0 ml of sulfuric acid in methanol and 25 ml of methanol were added and shaken. The solution was diluted and mixed with methanol to volume. 5.0 ml of a clear filtrate (Whatman No. 1 filter
paper) of the resulting solution was transferred to a 50.0 ml volumetric flask and diluted to volume using methanol. The reference standard solution was prepared using the same procedure as the sample preparation and both were analyzed using HPLC.

Similarly, MEB sample solutions were prepared from 20 tablets which were weighed and finely powdered. A portion of the powder equivalent to about 500 mg of MEB was transferred to a 100.0 ml volumetric flask. Fifty ml of formic acid was added and heated in a water bath at 50°C for 15 min. The solution was diluted with water to volume and it was mechanically shaken for 1 hour. 5.0 ml of a clear filtrate (Whatman No. 1 filter paper) of the resulting solution was transferred to a 25.0 ml volumetric flask and diluted to volume with the mobile phase. Finally, the diluted solution was mixed and filtered with a sintered glass filter (Millipore®, Darmstadt, Germany) with a pore size of 47 µm. The reference standard solution was prepared using the same procedure as the sample preparation, except the amount of MEB reference standard transferred was 25.0 mg and 10 ml of formic acid was added to the 100.0 ml volumetric flask. Finally, both the sample solutions and the reference were analyzed using HPLC.

For the assay of TNZ, three techniques were formulated by Basavaiah K, et al., 2005, giving reliable results of high accuracy and precision: potentiometry, spectrophotometry and HPLC. However, since USP 2007 uses the potentiometric method, TNZ was assayed using this pharmacopoeial method. Twenty tablets were weighed, finely powdered and a portion of the powder equivalent to 150 mg of TNZ was accurately weighed and dissolved in 25 ml of glacial acetic acid and titrated with 0.1 N perchloric acid standardized volumetric solution. The endpoint was determined potentiometrically using combined glass pH electrode (A1131B, Adwa Instruments, Szeged, Hungary).

Measured drug content was expressed as a percent of labeled claim (%lc). According to USP individual monographs for ALB and MEB tablets, they should contain not less than 90% and not more than 110% of the labeled amount based on USP 2007 requirements. Since there was no specific assay specification for TNZ tablets in USP, the same acceptance limits as used for ALB and MEB tablets were applied.

**Disintegration test**

With the assumption that the patient swallows with proper chewing, the disintegration test was not performed for MEB chewable tablets.

Disintegration test was thus carried out for ALB and TNZ tablet samples using USP <701> general monograph. Six tablets from each drug product were tested for disintegration times in 900 ml distilled water at 37±2 ºC using a disintegration apparatus A (PTZ-2E, PharmaTest, Germany) without
disk. The disintegration time was taken to be the time at which no tablet was left on the meshes of the apparatus under given set of conditions.

The general USP monograph for tablets requires that uncoated tablets, except for chewable tablets which are exempted from this test, disintegrate within 15 min.

**Dissolution study**

An *in-vitro* dissolution study was carried out for TNZ and ALB using a six vessels Dissolution Tester (Tian-Jin, RC-6D, China) following individual monographs in USP 2007. Dissolution study was not conducted for MEB samples since there were no sufficient samples left from the other tests.

For ALB tablets, it was performed using USP type II dissolution apparatus (paddle), while for TNZ apparatus type I (basket) was used. The dissolution medium was 900 ml of 0.1N hydrochloric acid at a temperature of 37±1°C and 50 rpm. Dissolution was carried out based on the pharmacopoeial defined timings for complete dissolution, which was 30 minutes for ALB and 120 minutes for TNZ.

Considering the rapid disintegration and dissolution, 4 ALB products (3 brands and 1 generic product) and 7 TNZ products (4 brands and 3 generic products) were purposefully selected to represent the products and tested for a complete release profile. At time intervals of 5 min for ALB and 10 min for TNZ, samples of 10.0 ml were withdrawn and replenished with an equal volume of fresh dissolution medium at the same temperature. Samples were filtered using Whatman No. 1 filter paper, suitably diluted and assayed using UV/Visible spectrophotometer (CECIL CE7200, England) at 350 nm and 317 nm, respectively. The calibration curves for ALB and TNZ were prepared as specified in individual monographs in USP 2007 by preparing six different concentrations from stock solution. The percent cumulative release was calculated and plotted against time of release.

For ALB tablets, the official tolerance limits according to USP individual monograph is that more than 80.0% should be released within 30 min, where as for TNZ tablets, more than 75.0% of the dose should be released within 120 min.

**Dosage uniformity**

The dose uniformity of tablets can be determined by two general approaches: the weight variation between the sampled tablets or the drug content uniformity. In this study, the weight variation test was performed as a means of quantifying uniformity of dose units since all tablet samples contain 100 mg or more active ingredient, i.e. all the drug products have a dose greater than 25 mg and a ratio of drug substance over finished product greater than 25% according to the specifications set in USP 2007 and Ph. Eur 2012. Ten tablets were selected at random, weighed all together using the analytical balance (Mettler Toledo, AL-204 AC, USA) and the average weight was calculated. Then, each tablet was weighed individually and the percentage deviation from average weight was
calculated. The acceptance value was calculated using the individual tablet weights and the assay result. The requirements for dosage uniformity are met if the acceptance value of 10 dosage unit is less than or equal to L1% (15.0). If the acceptance value is greater than L1%, further 20 tablets were taken and calculated for the acceptance value, which should then be below L2% (25.0).

**Friability test**
To evaluate the friability of the tablets of each drug product, a number of tablets, adding up to 6.5 g, were taken for MEB (unit mass < 650 mg), while 10 tablets were taken for ALB and TNZ (unit mass > 650 mg). Prior to the test, the tablets were carefully de-dusted and accurately weighed. The tablets were then placed in the drum of the friability test apparatus, PharmaTest Friabilator (PTF-20E, PharmaTest, Germany) and subjected to its tumbling action at 25 rpm for 4 min. After 4 minutes only intact tablets were once again de-dusted and weighed to determine the percentage weight loss by tablets due to mechanical action during test. If cracked, cleaved, or broken tablets were present in the tablet sample after tumbling, the sample was considered to fail the friability test.

According to USP general monograph, a maximum mean weight loss of not more than 1.0% is considered acceptable for most products based on USP 2007.

**Risk Analysis**
Risk analysis is a general quality tool which has its roots in engineering [27], but is now becoming a well-established tool in the pharmaceutical field as well. As such, ICH has devoted a separate guideline (Q9) to quality risk management, which is being embraced by pharmaceutical authorities [28]. Risk analysis, i.e. the estimation of the risk associated with the identified hazards, is an important part of this global risk management. Several quality risk management tools like FMEA (Failure Mode Effects Analysis) are available, as mentioned by ICH in Q9. Therefore, FMEA was used to evaluate the criticality of product quality attributes in this study. Criticality was evaluated using RPN, based on evaluations about the probability of occurrence of the failure (O), the severity of the failure (S) and the probability of not detecting the failure (D). These judgments are converted into numerical values using descriptive scales and finally combined in the RPN [29] by means of Equation (1):

\[
RPN = O \times S \times D
\]

Used scales for severity, occurrence and detectability of failure are presented in Tables 2 to 4 [30]. For severity ratings, five pharmaceutical experts in Belgium (4) and Ethiopia (1) were assigned to score it and the median score was taken. For occurrence, literature was reviewed for the three products (MEB, ALB and TNZ) in Africa and for other drugs in Ethiopia as there was no previous
quality study conducted for these three products in Ethiopia. In Nigeria, 48% of MEB samples contained amounts of active ingredient outside the appropriate assay limits [31]. Assay based pharmaceutical quality assessment in Kenya reported very poor quality for majority of marketed anthelmintic preparations [32]. Therefore, the highest occurrence score of 8 was assigned for assay. Studies conducted in Ethiopia indicated that occurrence of failure of identification, disintegration and friability tests are very low making the scores assigned to each of these failures to be 1 [18, 33, 34].

Table 2. Evaluation criteria and ranking system for the severity of effects.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Criteria: severity of effect</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazardous</td>
<td>Failure is hazardous, and occurs without warning. It suspends operation of the system</td>
<td>10</td>
</tr>
<tr>
<td>Serious</td>
<td>Failure involves hazardous outcomes and/or noncompliance with government regulations or standards</td>
<td>9</td>
</tr>
<tr>
<td>Extreme</td>
<td>Product is inoperable with loss of primary function. The system is inoperable</td>
<td>8</td>
</tr>
<tr>
<td>Major</td>
<td>Product performance is severely affected but functions. The system may not operate</td>
<td>7</td>
</tr>
<tr>
<td>Significant</td>
<td>Product performance is degraded. Comfort or convince functions may not operate</td>
<td>6</td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate effect on product performance. The product requires repair</td>
<td>5</td>
</tr>
<tr>
<td>Low</td>
<td>Small effect on product performance. The product does not require repair</td>
<td>4</td>
</tr>
<tr>
<td>Minor</td>
<td>Minor effect on product or system performance</td>
<td>3</td>
</tr>
<tr>
<td>Very minor</td>
<td>Very minor effect on product or system performance</td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>No effect</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Evaluation criteria and ranking system for the occurrence of failure.

<table>
<thead>
<tr>
<th>Probability of failure</th>
<th>Possible failure rates</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely high: failure almost inevitable</td>
<td>≥1 in 2</td>
<td>10</td>
</tr>
<tr>
<td>Very high</td>
<td>1 in 3</td>
<td>9</td>
</tr>
<tr>
<td>Repeated failures</td>
<td>1 in 8</td>
<td>8</td>
</tr>
<tr>
<td>High</td>
<td>1 in 20</td>
<td>7</td>
</tr>
<tr>
<td>Moderately high</td>
<td>1 in 80</td>
<td>6</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 in 400</td>
<td>5</td>
</tr>
<tr>
<td>Relatively low</td>
<td>1 in 2000</td>
<td>4</td>
</tr>
<tr>
<td>Low</td>
<td>1 in 15,000</td>
<td>3</td>
</tr>
<tr>
<td>Remote</td>
<td>1 in 150,000</td>
<td>2</td>
</tr>
<tr>
<td>Nearly impossible</td>
<td>≥1 in 1,500,000</td>
<td>1</td>
</tr>
</tbody>
</table>

Since 19.1% (8/42) of our MEB samples did not meet the pharmacopoeial acceptance criteria for dosage form uniformity, the probability of occurrence of this failure is moderately high and thus a score of 6 was assigned for its occurrence. For scoring the detectability, the scaling ranged from the low score assigned to the easiest detection to the highest score for the more difficult detection method. Friability can be detected through simple visual/weighing observation; hence, a score of 1
was assigned to its detectability. On the other hand, assay and dissolution studies involve quantitative tests, requiring fully equipped laboratory system and trained personnel. Therefore, detectability was scored to be 8 for each of these failure modes. Since identification requires field tests like color reactions and/or TLC, a score of 5 was assigned to detectability of identity failures.

<table>
<thead>
<tr>
<th>Detection</th>
<th>Criteria: likelihood of detection by design control</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute uncertainty</td>
<td>Design control does not detect a potential cause of failure or subsequent failure mode; or there is no design control</td>
<td>10</td>
</tr>
<tr>
<td>Very remote</td>
<td>Very remote chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>9</td>
</tr>
<tr>
<td>Remote</td>
<td>Remote chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>8</td>
</tr>
<tr>
<td>Very low</td>
<td>Very low chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>7</td>
</tr>
<tr>
<td>Low</td>
<td>Low chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>6</td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>5</td>
</tr>
<tr>
<td>Moderately high</td>
<td>Moderately high chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>4</td>
</tr>
<tr>
<td>High</td>
<td>High chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>3</td>
</tr>
<tr>
<td>Very high</td>
<td>Very high chance the design control will detect a potential cause of failure or subsequent failure mode</td>
<td>2</td>
</tr>
<tr>
<td>Almost certain</td>
<td>Design control will almost certainly detect a potential cause of failure or subsequent failure mode</td>
<td>1</td>
</tr>
</tbody>
</table>

**Desirability function**

Desirability function, just like risk analysis, is a quality tool first proposed by Harrington in 1965 for use in the optimization of quality of manufactured products. The approach has basic foundation in engineering [35, 36] and is widely adopted in the manufacturing industry.

The central idea of a desirability function is to create one ball-mark figure which is a composite number reflecting different response. This is done by mapping the value of each property/response onto a unit-less score in the range from zero to one based on the appropriateness (or desirability) of the property/response. Therefore, Derringer’s desirability function was applied for the assessment of the quality of the three pharmaceutical products (MEB, ALB and TNZ). The desirability function can be used to combine multiple responses into one response called the “overall desirability function” D, ranging between a value of 0 (one or more product characteristics are completely unacceptable) to 1 (all product characteristics are on target). This overall desirability function D is obtained from the
geometric mean of the individual desirabilities ($d_i$) which provide a way to assess the quality of one property. The formula to calculate the overall $D$-value is presented in Equation 2:

$$\sqrt[n]{\prod_{i=1}^{n} d_i^{p_i}}$$

(2)

In this equation, $p_i$ was the weight or relative importance assigned to the response. For this study, $n$ equals 4 since four characteristics were considered in the global evaluation of ALB and TNZ, while $n = 3$ for MEB since dissolution study was not performed. The advantage of calculating the geometric mean is that when one of the criteria has an unacceptable value, the overall product will be unacceptable as well. The highest global desirability value represents the product with the highest quality.

Individual desirability functions were defined for each of the quality attributes, based on a psychophysical scale and the results obtained from the FMEA quality assessment. Desirability function possessing values in the range $(0–1)$ classifies the conversion of the quantity value of a specific quality indicator into the assessment of the desirability (preference) of a certain condition of evaluated subject (pharmacopoeial quality of the three medicines). Among the specific ways to implement the desirability function for the corresponding estimation, a psychophysical scale of Harrington is chosen providing universal application. The scale served to establish the correspondence between physical and psychological parameters. All the numeric desirability values (0–1) of the measured parameters/quality attributes are regarded as physical parameters, while a purely subjective assessment of a researcher (e.g. excellent, good, acceptable, low, bad) to express degree of satisfaction are regarded as psychological parameters.

A rough estimation constructs a five–interval quality scale (Table 5) [37]. For assay and dissolution, a two-sided desirability function was used where it becomes zero at the lowest and upper limit. For identity and dosage form uniformity, a one-sided desirability function was used. Absence of API is assumed to be clinically completely undesirable and thus this point was assigned $d = 0$ where as 100%lc was assigned $d = 1$ (i.e. optimal desirability). Since the pharmacopoeial specification for assay is 90-110%lc for all the three products and the psychophysical Harrington’s scale of quality specifies desirability range from about 0.7 to 1.0 to be good, $d = 0.7$ was assigned for assay values of 90 and 110%lc. Moreover, $d = 0.3$ was assigned for both 70% and 130%lc, while $d = 0.01$ was assigned to 50% and 150%lc. The individual desirability function for assay was then defined as different linear sections of different slopes in the range of 100%lc to 90%lc (slope = 0.03), 90%lc to 70%lc (slope = 0.02) and from 70%lc to 50%lc (slope = 0.01). Similar but negative slopes were used for assay values greater than 100%lc, mirroring the under-dosing profile.
For dissolution, %drug release was considered. According to USP acceptance criteria (supporting information S1-2), ALB should release 80% within 30 minutes, while TNZ should release 75% within 120 minutes. However, BP sets acceptance criteria for both drugs at 70%. Therefore, \( d = 1 \) was assigned for 100% drug release, while \( d = 0.7 \) was assigned for the average 75% and 125% drug release for both ALB and TNZ. Moreover, \( d = 0.3 \) was assigned for both 50% and 150% drug release, while \( d = 0.01 \) was assigned to 40% and 160% drug release.

### Table 5. Modified psychophysical Harrington’s scale of quality and results of risk-based desirability function approach.

<table>
<thead>
<tr>
<th>#</th>
<th>Intervals in global desirability (D-global)</th>
<th>Quality, descriptive evaluation</th>
<th>Number of products in each quality scale (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.90-1.00</td>
<td>Excellent</td>
<td>14 (13%)</td>
</tr>
<tr>
<td>2</td>
<td>0.80-0.90</td>
<td>Good</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>3</td>
<td>0.70-0.80</td>
<td>Acceptable</td>
<td>38 (36%)</td>
</tr>
<tr>
<td>4</td>
<td>0.37-0.70</td>
<td>Low*</td>
<td>29 (27%)</td>
</tr>
<tr>
<td>5</td>
<td>0.00-0.37</td>
<td>Bad*</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

*Unacceptable qualities

For dosage uniformity, the relative standard deviation (RSD) was considered as response. According to Ph. Eur. (2012), RSD should be not more than 2%; and thus \( d = 1 \) was assigned for RSD = 0% while \( d = 0.7 \) for RSD = 2%. Following Harrington’s scale, \( d = 0.3 \) was assigned for RSD of 6% and \( d = 0.01 \) for RSD of 15%; while for RSD = 25%, \( d \) was assigned to be 0.

For identity, \( d = 1.0 \) was assigned for those complying with pharmacopoeial specifications for identity and \( d = 0 \) for those which do not comply.

### Data analysis

Data entry and analysis was carried out using Statistical Package for Social Sciences software (version 16.0 for windows; SPSS). The assay was carried out in triplicate and data were expressed as mean values. The Fisher exact test was used to test the association of the binary quality attributes with the country of origin (5 origins), collection sites (7 cities) and drug outlets (3 types). A more detailed statistical data analysis, based on the fixed effects model with different response variables (product quality attributes) and different categorical covariates derived from our sampling strategy questions was done. FMEA was used to assess the criticality of the quality risks associated with each quality attribute and Derringer’s desirability function was applied to evaluate quality of the products.
3. RESULTS

Quality of the investigated products

A total of one hundred and six samples of MEB, ALB and TNZ were collected between January and March 2012 in seven major cities that represent most parts of the country considering pharmaceutical market and geographic areas. The samples had been collected from 38 premises (wholesales, pharmacies and drug stores). Of these, 42 samples were MBZ, 25 samples were ALB and 39 were TNZ samples. The origin (place of manufacturing) of samples was domestic and foreign (China, India, Korea, and Cyprus). Domestic products constituted 45.3% (48/106), followed by Indian products with 26.5% (28/106). All samples had the intended active ingredient as demonstrated by the positive identification tests. No gross mislabeling (incorrect, inadequate or incomplete identification) was observed for the samples. However, the quantitative laboratory experiments indicated that 45.3% (48/106) of the samples did not meet the expected pharmacopoeial quality specifications: 45.2% (19/42) MEB, 48.0% (12/25) ALB and 43.6% (17/39) TNZ samples. The results of the different quality control tests of the samples are presented in Table 6 and are detailed below.

Table 6. Pharmacopoeial quality test results by product.

<table>
<thead>
<tr>
<th>Product and strength (mg)</th>
<th>Assay</th>
<th>Dissolution</th>
<th>Dosage uniformity</th>
<th>Friability</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALB (400)</td>
<td>8% (2/25)</td>
<td>42% (8/19)</td>
<td>0% (0/25)</td>
<td>20% (5/25)</td>
<td>48% (12/25)</td>
</tr>
<tr>
<td>MEB (100)</td>
<td>45% (19/42)</td>
<td>-</td>
<td>19% (8/42)</td>
<td>7% (3/42)</td>
<td>45% (19/42)</td>
</tr>
<tr>
<td>TNZ (500)</td>
<td>26% (10/39)</td>
<td>18% (7/39)</td>
<td>0% (0/39)</td>
<td>8% (3/39)</td>
<td>44% (17/39)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>29% (31/106)</strong></td>
<td><strong>26% (15/58)</strong></td>
<td><strong>7% (8/106)</strong></td>
<td><strong>10% (11/106)</strong></td>
<td><strong>45% (48/106)</strong></td>
</tr>
</tbody>
</table>

- Not performed.

Assay

The assay values for MEB drug products ranged from 68.6 to 132.9 %lc (mean: 106.2%), while that of ALB ranged from 87.1 to 111.0 %lc (mean: 98.6%). For TNZ drug products, the assay values ranged from 86.1 to 120.6 %lc (mean: 105.7%). Assay test results by product brand is presented in Table 7, while a box plot of assay by product type, country of origin, supply chain and place of collection is indicated in Figure 2.

This study revealed that 29.2 % (31/106) of samples did not meet the pharmacopoeial acceptance specification for the assay, and thus are formally classified as substandard medicines [13]. A high failure rate, 45.2% (19/42) was found for MEB tablets followed by TNZ with failure rate of 25.6 % (10/39) and 8.0% (2/25) of ALB samples. From those 31 samples failing to meet the official
specification limit for assay, 80.7% (25/31) of the samples were over-dosed and 19.4% (6/31) were under-dosed. MEB samples showed the highest variation for assay test with a relative standard deviation (RSD) of 12.5%, followed by TNZ and ALB with RSD 6.7% and 4.8% of the labeled amount, respectively. Considering the time left to expiry date, all ALB samples expired in 2013, while for MEB and TNZ, the expiry date was longer, i.e. 2015/16, which can explain the difference in assay values between the 3 drug product classes.

The assay results reveal that the majority of the failed samples contain too much active ingredient that may be introduced intentionally during production (i.e., overages applied). However, as a

---

Table 7. Assay test results by product type.

<table>
<thead>
<tr>
<th>Drug products (n)</th>
<th>Brands (n)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEB (42)</td>
<td>M1 (14)</td>
<td>98.6%</td>
<td>131.1%</td>
<td>108.6%</td>
<td>9.7%</td>
<td>106.6%</td>
</tr>
<tr>
<td></td>
<td>M2 (14)</td>
<td>68.6%</td>
<td>132.9%</td>
<td>107.6%</td>
<td>17.2%</td>
<td>107.7%</td>
</tr>
<tr>
<td></td>
<td>M3 (7)</td>
<td>81.0%</td>
<td>109.4%</td>
<td>95.0%</td>
<td>10.3%</td>
<td>93.0%</td>
</tr>
<tr>
<td></td>
<td>M4 (5)</td>
<td>98.7%</td>
<td>115.6%</td>
<td>105.8%</td>
<td>7.4%</td>
<td>102.2%</td>
</tr>
<tr>
<td></td>
<td>M5 (1)</td>
<td>121.1%</td>
<td>121.1%</td>
<td>121.1%</td>
<td>NA</td>
<td>121.1%</td>
</tr>
<tr>
<td></td>
<td>M6 (1)</td>
<td>118.7%</td>
<td>118.7%</td>
<td>118.7%</td>
<td>NA</td>
<td>118.7%</td>
</tr>
<tr>
<td>Sub total</td>
<td></td>
<td>68.6%</td>
<td>132.9%</td>
<td>106.2%</td>
<td>13.3%</td>
<td>106.0%</td>
</tr>
<tr>
<td>ALB (25)</td>
<td>A1 (1)</td>
<td>92.0%</td>
<td>92.0%</td>
<td>92.0%</td>
<td>NA</td>
<td>92.0%</td>
</tr>
<tr>
<td></td>
<td>A2 (9)</td>
<td>96.7%</td>
<td>108.0%</td>
<td>99.2%</td>
<td>3.5%</td>
<td>97.9%</td>
</tr>
<tr>
<td></td>
<td>A3* (6)</td>
<td>94.2%</td>
<td>102.4%</td>
<td>97.5%</td>
<td>3.1%</td>
<td>96.4%</td>
</tr>
<tr>
<td></td>
<td>A4 (2)</td>
<td>99.5%</td>
<td>103.3%</td>
<td>101.4%</td>
<td>2.7%</td>
<td>101.4%</td>
</tr>
<tr>
<td></td>
<td>A5 (1)</td>
<td>104.0%</td>
<td>104.0%</td>
<td>104.0%</td>
<td>NA</td>
<td>104.0%</td>
</tr>
<tr>
<td></td>
<td>A6 (3)</td>
<td>87.1%</td>
<td>100.0%</td>
<td>95.3%</td>
<td>7.1%</td>
<td>98.8%</td>
</tr>
<tr>
<td></td>
<td>A7 (1)</td>
<td>96.3%</td>
<td>96.3%</td>
<td>96.3%</td>
<td>NA</td>
<td>96.3%</td>
</tr>
<tr>
<td></td>
<td>A8 (1)</td>
<td>94.3%</td>
<td>94.3%</td>
<td>94.3%</td>
<td>NA</td>
<td>94.3%</td>
</tr>
<tr>
<td></td>
<td>A9 (1)</td>
<td>111.0%</td>
<td>111.0%</td>
<td>111.0%</td>
<td>NA</td>
<td>111.0%</td>
</tr>
<tr>
<td>Sub total</td>
<td></td>
<td>87.1%</td>
<td>111.0%</td>
<td>98.6%</td>
<td>4.8%</td>
<td>97.9%</td>
</tr>
<tr>
<td>TNZ (39)</td>
<td>T1 (3)</td>
<td>86.1%</td>
<td>99.1%</td>
<td>94.7%</td>
<td>7.5%</td>
<td>99.1%</td>
</tr>
<tr>
<td></td>
<td>T2 (7)</td>
<td>107.7%</td>
<td>112.0%</td>
<td>108.9%</td>
<td>2.1%</td>
<td>107.7%</td>
</tr>
<tr>
<td></td>
<td>T3* (5)</td>
<td>99.1%</td>
<td>112.0%</td>
<td>104.2%</td>
<td>4.7%</td>
<td>103.4%</td>
</tr>
<tr>
<td></td>
<td>T4* (9)</td>
<td>99.1%</td>
<td>107.7%</td>
<td>102.4%</td>
<td>2.9%</td>
<td>103.4%</td>
</tr>
<tr>
<td></td>
<td>T5 (8)</td>
<td>99.1%</td>
<td>120.6%</td>
<td>113.6%</td>
<td>7.6%</td>
<td>114.1%</td>
</tr>
<tr>
<td></td>
<td>T6 (5)</td>
<td>94.8%</td>
<td>103.4%</td>
<td>101.6%</td>
<td>3.9%</td>
<td>103.4%</td>
</tr>
<tr>
<td></td>
<td>T7* (2)</td>
<td>107.7%</td>
<td>107.7%</td>
<td>107.7%</td>
<td>0.0%</td>
<td>107.7%</td>
</tr>
<tr>
<td>Sub total</td>
<td></td>
<td>86.1%</td>
<td>120.6%</td>
<td>105.7%</td>
<td>7.0%</td>
<td>103.4%</td>
</tr>
<tr>
<td>Total (106)</td>
<td></td>
<td>68.6%</td>
<td>132.9%</td>
<td>104.2%</td>
<td>10.1%</td>
<td>103.4%</td>
</tr>
</tbody>
</table>

*Generic products; SD= Standard deviation; NA = Not applicable
General principle, use of an over-dose of a drug substance to compensate for loss during manufacture or degradation during a product’s shelf life to extend its shelf life, is discouraged [38].

Figure 2. Box plot for assay versus (a) product type, (b) origin, (c) premises/supply chain and (d) place of collection. Numbers are given between the brackets.

Disintegration test
In this study, all tablet samples met the official requirement for disintegration time test.

Dissolution test
As shown in Table 6, from 19 ALB and 39 TNZ samples tested for their in-vitro dissolution, 42.1% (8/19) of ALB and 17.9% (7/39) of TNZ samples failed to meet the official tolerance limits. There is a significant difference between countries of origin with respect to the in-vitro dissolution profile, with all samples manufactured in Ethiopia (19/19) meeting the official tolerance limit and 25.9% (15/58) failure rate observed for the imported products. From the 11 products (4 ALB and 7 TNZ)
purposefully selected for the release profile study, two brands of the four ALB products released more than 80.0 %lc in 30 minutes while a brand and one generic ALB products failed to comply with the release profile. Except for the generic product, a fast release was observed from all ALB products in which more than 70.0 %lc was released within 10 minutes as presented in Figure 3a. All four TNZ brands and three generic products released more than 75.0 %lc of the dose within 120 minutes as indicated in Figure 3b.

![Figure 3a](image1.png) ![Figure 3b](image2.png)

**Figure 3.** Comparative in-vitro release studies of (a) four different products of albendazole (ALB) tablets and (b) different products of tinidazole (TNZ) tablets. All data points presented are mean values of triplicate experiments (n=3) and error bars indicate standard deviations. Percent drug release should be between 70 and 130% within 30 min (ALB) and 120 min (TNZ).

**Dosage uniformity**
Dosage uniformity is measured to ensure a constant dose of drug between individual dosage forms. All ALB and TNZ samples were in line with pharmacopoeial acceptance criteria for dosage uniformity, but 19.1% (8/42) of MEB samples did not meet these specifications as indicated in Table 6.

**Friability test**
A relatively high failure rate (20%) of ALB samples followed by TNZ (7.7%) and MEB (7.1%) was observed in the present study. Overall 10.4% (11/106) of samples failed to meet the friability test. The higher friability for ALB products might be related to the rapid disintegration and dissolution of these products.

**Risk analysis**
The results of the RPN values after scores assigned for severity, occurrence and detectability of the failure mode are presented in Table 8. In the quality attributes subjected to FMEA, a total of 5 failure
modes with RPN scores ranging from 2 to 512 were identified. Risk analysis showed that assay (RPN = 512) is the most critical quality attribute followed by dissolution (RPN = 336) and dosage uniformity (RPN 144). Friability was found to be the quality attribute of the least concern according to FMEA analysis applied to product quality assessment.

**Derringer’s desirability function**

The individual desirability values assigned to the different segments were fitted to the segmented linear model as indicated in Figure 5.

![Figure 5. Linear desirability functions: (a) assay (% label claim), (b) dissolution (% drug release), (c) dosage uniformity (% RSD) and (d) identity (compliance to specification).](image)
For each medicine analyzed for the retained 4 quality attributes (assay, dissolution, dosage form uniformity and identity), a global D was finally calculated using the above mentioned d-functions and evaluated using the psychophysical Harrington’s scale of quality as presented in Table 5. According to this scale, it was revealed that 13.2% (14/106) of the products were excellent, while 22.6% (24/106) were good and 35.8% (38/106) were of acceptable quality. Thirty products (28.3%) were found to be of unacceptable quality (low and bad). Moreover, the distribution of the D-values among the investigated products is presented in Figure 6.

Table 8. Failure mode effects analysis (FMEA) for the different MEB, ALB and TNZ drug product quality attributes.

<table>
<thead>
<tr>
<th>#</th>
<th>CQA</th>
<th>Failure mode</th>
<th>Failure effects</th>
<th>S</th>
<th>O</th>
<th>D</th>
<th>RPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Identity</td>
<td>No (intended) active ingredient in the sample or mislabeling (incorrect, inadequate or incomplete identification)</td>
<td>Treatment failure, death due to untreated disease</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Assay</td>
<td>Under-dose, over-dose</td>
<td>Treatment failure, toxicity due to over-dose, drug resistance due to underdose</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration</td>
<td>Inability to sufficiently disintegrate within the specified time period</td>
<td>No or poor absorption and bioavailability thus leading to treatment failure and resistance</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Dissolution</td>
<td>Inability to sufficiently dissolve within the specified time period</td>
<td>Poor absorption and bioavailability thus leading to treatment failure and resistance</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>336</td>
</tr>
<tr>
<td>5</td>
<td>Friability</td>
<td>Tablets weight loss due to distribution or any other logistic related factors</td>
<td>Sub-optimal therapy due to loss of the active ingredient</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

CQA: critical quality attributes; S: severity; O: occurrence; D: detectability and RPN: risk priority number

4. DISCUSSION

To address the subject of quality of medicines, different internationally accepted semantics and definitions are used. In this study, the semantics “poor quality” was used due to the following reasons:
1) We did not find obvious features which suggest that the investigated samples were counterfeit, falsified and/or unregistered; although we did not explicitly search for counterfeits, falsified and/or unregistered medicines. Therefore, without a detailed investigation involving the manufacturer, distribution-chain and health authorities, it is impractical to rule out this perspective.

2) Poor quality can have good manufacturing practice (GMP) and/or good distribution practice (GDP) reasons, and as such, we do not differentiate explicitly between GMP-substandard (at manufacturer) versus degraded substandard (while being good quality at manufacturer). In this study, it was not practical to easily differentiate if poor quality was due to weak GMP or rather inadequate GDP. Moreover, the WHO as well as US-IOM (institute of medicine), and recent expert opinions [39] do not make this distinction and prefer to use the term ‘substandard’ to address both. Finally, quality does also include the intrinsic stability of the medicine (a very important aspect in tropical climates), which is a function of its composition, quality of ingredients/packaging and manufacturing process [40].

![Figure 6. Distribution of D-values among the investigated products.](image)

3) Even though the term ‘substandard’ literally means “under the standard”, it is obviously related to a legally required specification mostly interpreted in the national regulations in the approved marketing registration file and/or national pharmacopoeia/compendia, where some still use other quality standards, e.g. Ph.Int. or USP-MC. They all differ not only in quality attributes and methods, but also in acceptance criteria as well. As in Low and Middle Income Countries (LMIC),
the legally required standards are often absent and incomplete. Therefore, we prefer to use “poor quality” over “substandard”, as for the investigated products, we were not aware of the legally binding national Ethiopian quality standards. Moreover, we did not only use more international quality specifications, but also wanted to introduce the Taguchi-concept in our quality-evaluation, thus (to some extent) avoiding the “standard” issue, with an on-off decision, and replacing it by a quantitative quality number.

4) While other semantics like (S)SFFC, fake, and the like are sometimes used, we believe in accordance with EMA, US-IOM, and recent expert opinions, that for the purpose of this study, a simple 2-dimensional division between falsified and substandard will be sufficient.

In this study, we conducted quality evaluations based on two different approaches: the conventional viewpoint (dichotomous decision based on arbitrarily pharmacopoeial acceptance limits) and the risk-based desirability function approach. The conventional perspective is based on the acceptance criteria set in general and individual monographs of different pharmacopoeias and guidelines, while the desirability function approach is based on quality-by-design (QbD) and risk-based principles whereby clinical relevance is a key factor. A medicine can have many different quality attributes, which are certainly not equally important, i.e. each quality attribute has a different criticality for the clinical use of the medicine. This ICH-recommended risk-based approach is derived from the Taguchi quality philosophy, where any deviation from the optimal point is considered as a less optimal situation and there is no dichotomous decision.

Therefore, this study reports not only the percentage compliant with the generally accepted pharmacopoeial specification limits for each of the quality attributes using the conventional, dichotomous approach, but also derived a global quality number which encompasses the clinical importance of the different quality attributes. This clinical importance, i.e. criticality or risk if deviating from the optimum, was assessed by quality risk tools: within FMEA, one uses the risk priority number (RPN) to estimate this risk.

**Conventional quality of investigated medicines**

In general, the prevalence of poor quality medicines was the highest for ALB tablets (48.0%, 95% CI: 28.4 to 67.6), followed by MEB (45.2%, 95% CI: 30.2 to 60.3) and TNZ (43.6%, 95% CI: 28.0 to 59.2) tablets (Table 6). Overall, 45% (48/106) of the analyzed drug samples failed to meet the official tolerance limits for assay, dissolution, friability and uniformity of dose.

A similar survey conducted on anti-malarial drugs in Senegal, Madagascar and Uganda identified 44%, 30%, and 26% substandard anti-malarial drugs, respectively [41]. Assay and dissolution profile study for anti-malarial samples conducted in south-east Nigeria reported 37% substandard medicines [42].
Assay based pharmaceutical quality assessment in Kenya reported that many anthelmintic preparations marketed in Kenya were of very poor quality [32].

The probable causes for the presence of poor quality medicines in developing countries like Ethiopia might be due to poor storage conditions, insufficient quality assurance, poor compliance with good manufacturing practice standards, lack of scientific expertise in manufacturing sector, limited technical capacity and insufficiently well developed regulatory system to evaluate and take action to solve the problems related to drug quality [43].

From those drug samples collected from pharmacy, about 51.1% (24/46) failed while 46.9% (23/55) and 20.0% (1/5) were the failure rates for those collected from drug store and wholesale, respectively. Even though the sample size was small to generalize, there was significant difference in the pharmacopoeial quality parameter of medicines between the country of origin (P<0.05) but there was no significant association for place of collection and outlets, P>0.05 as presented in Table 9 and Figure 2.

Table 9. Association between the test results and areas of collection, types of drug outlets, and countries of origin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Registered quality failure</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Place of collection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assosa (14)</td>
<td>4/14 (28.6%)</td>
<td></td>
</tr>
<tr>
<td>Hawasa (19)</td>
<td>3/19 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>Addis Ababa (20)</td>
<td>10/20 (50.0%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Jimma (16)</td>
<td>10/16 (62.5%)</td>
<td></td>
</tr>
<tr>
<td>Adama (18)</td>
<td>9/18 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>Bahirdar (11)</td>
<td>6/11 (54.5%)</td>
<td></td>
</tr>
<tr>
<td>Mekele (8)</td>
<td>6/8 (75.0%)</td>
<td></td>
</tr>
<tr>
<td>Total (106)</td>
<td>48/106 (45.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Drug outlets</strong></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>Wholesale (5)</td>
<td>1/5 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>Pharmacy (46)</td>
<td>24/46 (52.2%)</td>
<td></td>
</tr>
<tr>
<td>Drug stores (55)</td>
<td>23/55 (41.8%)</td>
<td></td>
</tr>
<tr>
<td>Total (106)</td>
<td>48/106 (45.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Ethiopia (48)</td>
<td>21/48 (43.6%)</td>
<td></td>
</tr>
<tr>
<td>India (28)</td>
<td>11/28 (39.3%)</td>
<td></td>
</tr>
<tr>
<td>Cyrus (15)</td>
<td>8/15 (53.3%)</td>
<td></td>
</tr>
<tr>
<td>China (6)</td>
<td>6/6 (100.0%)</td>
<td></td>
</tr>
<tr>
<td>Korea (9)</td>
<td>2/9 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>Total (106)</td>
<td>48/106 (45.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Regarding the collection areas, a high failure rate was observed for samples collected from Addis Ababa, Jimma and Adama areas. Since these areas are commercial centers due to their geographic

144
location, it requires special attention by the regulatory offices to control the circulation of these anthelmintic medicines to combat poor quality medicines circulation.

All analyzed samples contained the intended active ingredient. Even though a single case of API-absent medicine is unacceptable, the finding of this study was good as compared to other studies, e.g. in Cambodia (4.2%) [44]. However, 29.2% (31/106) of the samples did not comply with the pharmacopoeial acceptance criteria for assay. Of the MEB samples, 45.2% were found to be of poor quality with respect to assay as per the official tolerance limit. This result is in agreement with the study conducted in Nigeria’s pharmacies in which 48% samples of MEB did not comply with set pharmacopoeial limits [31]. On the other hand, ALB samples showed relatively better compliance but still unacceptable as 8.0% did not meet the official acceptance limit for assay. In general, from those drug samples which failed assay test, 19.4% (6/31) were under-dosed. One of the contributing factors for the development of drug resistance is under-dosing due to poor quality medicines [45].

Uniformity of dosage unit is defined as the degree of uniformity in the amount of active substance among individual dosage units. Content uniformity depends on a number of formulations and manufacturing processes, hence it is obviously unrealistic to presume that every unit contains exactly the same amount of the active ingredient as indicated on the label. Therefore, pharmacopoeial standards and specifications have been established to provide generic limits for allowable variations for the active ingredients in single dosage units considering fitness-for-use and production capability considerations [46]. It was previously reported that (single dose) ALB is more efficacious against hookworm than (triple dose) MEB [47], which may partly be explained by our quality results revealing that all ALB and TNZ samples fulfill the acceptance criteria for dosage uniformity while 19.1% (8/42) of MEB samples did not meet these pharmacopoeial acceptance criteria.

Friability test is conducted to check whether the weight loss during handling is within 1.0% loss specification limit. As indicated in Table 6, 5 ALB, and 3 MEB and 3 TNZ tablet samples failed the pharmacopoeial acceptance criteria of friability. The percent weight loss for all the drug samples failing the specification criteria ranges between 2.2 to 6.0%, where the largest weight loss was registered from a MEB tablet sample. Taking into consideration the single dose regimen and the already substandard drugs with content less than 90 %lc, this maximum weight loss from friability study by MEB sample could further pose more risk of drug resistance leading to treatment failure than the other two drugs, ALB and TNZ.

In the present study, since all the drug samples tested for disintegration have met the pharmacopoeial acceptance criteria, there is no risk associated with disintegration as a quality attribute. However, 42% of the ALB samples and 18% of the TNZ samples which were tested for dissolution have been found to be out of the pharmacopoeial specification limit. For low solubility
drugs, raw material and process variables could have impact on clinical safety and efficacy through their effects on dissolution. Therefore, the risk of clinical failure is higher for ALB than TNZ as more delayed dissolution was observed, which could be due to changes in the drug substance particle size, failure to control granulation, and increased level of binder in the formulation [48].

The information available on the effectiveness of various BZs derivatives (e.g. ALB and MEB) is somewhat inconsistent [49, 50]. Thus the observations of different therapeutic outcomes have been to some extent attributed to the different polymorphs with different dissolution rates and anthelmintic activities. Solid-state properties play crucial role in dissolution rate and solubility, especially when different polymorphs are involved affecting the in-vivo performance of the drugs [51-54]. For example, MEB exists as polymorphs and solvates in the solid state. Of particular importance is the difference in the physicochemical properties of the three known polymorphs A, B, and C. The polymorphic forms of MEB display significant differences in solubility and therapeutic efficacy and form C is preferred clinically due to its optimal bioavailability and reduced toxicity. This is important because polymorph A has no anthelmintic activity alone or when present above 30% in polymorphic mixtures. Literature indicate that at temperatures typically found in countries located in ICH climatic zones III (hot and dry) and IV (hot and humid) trace amounts of form A in tablets significantly accelerate the transformation of the clinically active polymorph C to form A. This transformation significantly reduces the shelf lives and the dissolution rates of these tablets [55].

ALB also exhibits some polymorphic forms by forming solvated crystals. Each of these crystals, including the un-solvated form, may exhibit all the aspects of polymorphism. However, solid state characterization of ALB indicated that both forms are physically quite stable [51]. A literature report indicated that TNZ also exhibits crystal polymorphism [56].

Regarding the use of ALB or MEB, specific attention should be given to the dose appropriate for infants (12 months and less). Apart from the likelihood of both prevalence and intensity being relatively low in infants in areas where soil-transmitted helminthiasis is endemic, there are questions of efficacy and safety when using an anthelmintic drug in very young children [57]. Some studies reveal that the no observed effect level/no observed adverse effect level (NOEL/NOAEL) for ALB is 7 mg/kg/day and that of MEB was found to be 7.8 and 8.4 mg/kg/day in males and females, respectively in experimental animals [58]. Taking the studied ALB tablets, it is possible to assess the associated risk due to the overdosed assay values. The standard treatment guideline for Ethiopia recommends 400 mg tablet as a single dose for treatment of different helminths infections [58]. Assuming an average body weight of 70 kg (body mass index: 23 and height: 175 cm), the NOEL/NOAEL value for ALB can be calculated to be 490 mg per day (taking a safety factor of 1), equivalent with 122.5 %lc for a 400 mg tablet. All the assay values for ALB drug products were found to be less than or equal to 111.0 %lc, indicating absence of clinically significant risk for the ALB
overdosed formulation related to adverse effects. For MEB, since the treatment guideline recommends 200 mg per day [59] and the NOEL/NOAEL value is much higher, the over-dose in the assay values is not a direct clinical concern related to adverse effects.

The assay distribution of the analyzed TNZ samples was found to be from 86.1 to 120.6 %lc. Considering the 2 g single dose regimen of TNZ for treatment of giardiasis and the high level NOEL/NOAEL value of 150 mg/kg together with the relative clinical safety of TNZ, the over-dose in the assay values is also not a direct clinical concern related to the adverse effects.

Under-dosing, which could be caused by degradation due to inappropriate storage conditions, might pose toxicity risks due to the degradant impurities. It can be one of the risk factors for the development of anthelmintic resistance. Sub-optimal regimens are the rule in human treatment: anthelmintics are administered in single doses that never achieve 100% efficacy. Taking into account the limited efficacy of single dose anthelmintic treatments, the currently recommended regimens could constitute a significant contributing factor to the development of anthelmintic resistance in STH [60]. In addition to the single dose regimen, the substandard drugs with content less than 90 %lc, could further exacerbate the problem of drug resistance leading to treatment failure. Therefore, the risk of development of drug resistance to MEB is higher than the other two drugs, ALB and TNZ since four of the six under-dosed substandard drug samples were MEB.

**Risk-based approach to medicines quality**

FMEA is a well-known assessment tool used to identify the critical components most likely to cause failures and to enhance system reliability, through the development of suitable corrective and preventive actions (CAPAs) [61]. Typically, the criticality is evaluated either with the criticality number (CN), or with the risk priority number (RPN). Although the CN is considered more consistent and accurate, the RPN approach is generally preferred, especially for its easiness of use [62], where the higher RPN values indicate the criticality of the quality attribute.

**The desirability function and its application in evaluation of quality of medicines**

Optimizing parameters is a critical issue during the development of any method and/or product. A special set of functions called desirability functions have been used in optimizing methods [63, 64] and products characteristics [65, 66]; but the application of such desirability functions for the assessment of the quality of pharmaceutical products is new.
The overall desirability function $D$ is obtained from the individual desirabilities ($d_i$) using Equation 2. It can provide a way to assess the quality according to one property, the overall $D$-value. By mapping all properties onto a desirability scale between 0 and 1, the individual desirability scores due to multiple properties may be easily combined as a geometric mean even if the properties have different scales or units of measurement [67].

In the calculation of the overall $D$-value using Equation 2, $p_i = 3$ was used for assay since quality risk associated to it was found to be more important (RPN = 512). Similarly, $p_i = 2$ was used for dissolution since the risk associated with dissolution was of more concern (RPN = 336) than others. For each of identity and dosage uniformity, $p_i = 1$ was assigned. The risk assessment revealed that friability was not critically important with calculated RPN value of only 2 and thus was not considered for the desirability study.

The risk analysis conducted indicated that the failure effects due to the failure modes (non-complying quality attributes) was found to be almost similar for the three products analyzed. For example, for all, the over-dose in the assay values was evaluated to be not a direct clinical concern related to the adverse effects. Moreover, since all the three drugs are in BCS class II [26], dissolution is equally a concern. Therefore, the same Derringer’s desirability function was applied to all the drug products.

In general, comparing the two quality evaluation approaches, it is reported that 29.2% of the samples were of poor quality when using the pharmacopoeial method of quality evaluation, while it is 28.3% using the new innovative risk-based desirability function approach. Even though it seems that there is no discrepancy between the results of the conventional and D-function approach, we still want to argue that the D-approach provides more weight to the clinically more critical quality attributes and thus fit-for-purpose in resource-limited economies. Resources could thus be prioritized and reliable decisions can be made on the available data using only the clinically more critical quality attributes (assay and dissolution) than the less critical ones (friability and disintegration tests). Moreover, the new QbD and risk-based approach will less heavily penalize marginal out-of-specification medicines, and therefore, we believe it is especially important for poor-resource countries.

5. CONCLUSIONS

The study indicated that all sampled products (MEB, ALB and TNZ) did contain the stated active ingredient, but poor quality products were identified in all three medicines and collection sites in the country due to non-compliant assays, inadequate drug release of required dose or toxicity concerns due to over-dosage of some of the medicines containing higher level of active ingredient. Over-dose
in the assay values of the three studied drugs is not a direct clinical concern related to adverse effects where as under-dosing constituted one of the risk factors for the development of resistance.

The study further identified the most critical quality attributes in product quality assessment using FMEA risk-based quality evaluation of the three drugs where assay was found to be the most critical quality attribute with highest RPN. Moreover, it was revealed that Derringer’s desirability function can be applied to pharmaceutical quality assessment using Psychophysical Harrington’s scale of quality where products could be classified into excellent, good, acceptable, low and bad quality.

Our study suggests policy strategies of containing the problems related to poor quality medicines using this proactive risk-based and desirability function approaches in nation-wide surveillance of the quality of medicines circulating in their respective markets. Furthermore, other possible strategies for containing the problem of these poor quality medicines are e.g.:

- strengthening the capacity of drug regulatory authorities for quality assurance and quality control activities;
- harmonization and regional sharing of information about manufacturing and distribution quality;
- enforcement of regulations and legal prosecutions;
- empowerment and capacity building of medicines inspectors;
- continuous inspection and monitoring of the different levels of medicines supply chain;
- continuous and sustainable product quality surveillance studies with strong monitoring and evaluation activities

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CHAPTER VI

PHARMACEUTICAL REGULATORY FRAMEWORK IN ETHIOPIA: CRITICAL EVALUATION OF ITS LEGAL BASIS AND IMPLEMENTATION

“It is very expensive to give bad medical care to poor people in a rich country.”

Paul Farmer
(American anthropologist and physician)

Parts of this chapter were published:

ABSTRACT

Effective and enforceable national regulations describing the manufacture and (re)packaging, export and import, distribution and storage, supply and sale, information and pharmaco-vigilance of medicines are required to consistently ensure optimal patient benefit. In Ethiopia, a comprehensive regulation of the pharmaceutical market was introduced in 1964, and is currently regulated by the “Food, medicine and health care Administration and Control Proclamation No.661/2009”. The mere existence of this legal framework does not guarantee complete absence of illegal, substandard and falsified products as well as illegal establishments in the pharmaceutical chain. Therefore, the objective of the research is to assess the pharmaceutical regulatory system in Ethiopia and to reveal possible reasons for deficiencies in the pharmaceutical chain. The study revealed that Ethiopia does have a written national drug policy upon which the medicines regulatory proclamation 661/2009 is based. According to this proclamation, the Ethiopian Food, Medicines and Healthcare Administration and Control Authority is mandated to execute the regulatory activities as per the council of ministers regulation 189/2010. The legal framework for pharmaceutical regulation of Ethiopia was found to fulfill all the medicines regulatory functions potentially enabling to combat illegal, substandard and falsified medicines and illegal establishments. It was revealed that there exist illegal sources for medicine in the pharmaceutical market. The main reasons for their existence were regulatory factors including weak regulatory enforcement (64.5%), lack of informal market control (60.8%), weak port control (50.0%), and poor cooperation between executive bodies (39.6%); and resource constraint (27.8%), which is an institutional factor.
Main focus in this chapter:

- To critically review the legal basis for pharmaceutical regulation in Ethiopia.
- To assess the implementation of the pharmaceutical regulatory frame in Ethiopia.

1. INTRODUCTION

The fundamental purpose of medicine regulation is the protection of public health and to ensure that medicines on national markets and in international commerce are safe, effective and of good quality, and used in accordance with good practices. Medicines are important aspects of public health and must be available and accessible to the public. To improve access to medicines, good governance is crucial and contributes to health systems strengthening. Good governance in the pharmaceutical sector refers to the formulation and implementation of appropriate policies and procedures that ensure the effective, efficient and ethical management of medicine regulation, in a manner that is transparent, accountable and follows the rule of law [1-4].

Since the mid-1930s, many new pharmaceutical products have flourished and trade in the pharmaceutical industry has taken on international dimensions. However, the circulation of toxic, substandard and counterfeit drugs on the national and international market has increased. This is mainly due to ineffective regulation of production and trade in pharmaceutical products in both exporting and importing countries. The use of these poor quality medicines may also threaten the
health and lives of patients [5]. WHO estimates that from one million deaths that occur from malaria annually, 200,000 would be avoidable if the medicines available were effective, of good quality and used correctly. A study conducted in South-East Asia in 2001 revealed that 38% of 104 anti-malarial drugs on sale in pharmacies did not contain any active ingredient and resulted in a number of preventable deaths [6]. Moreover, inefficiencies in medicines regulatory system can delay entry of needed medicines in a market; hence, a barrier to access for users and to the profits and growth of the pharmaceutical business [7].

According to WHO, about 20% of countries have well-developed and operational medicines regulation. Of the rest, approximately half have regulation of varying capacity and level of development, and 30% have either no or very limited medicines regulation; revealing that many low-income countries cannot ensure safety, efficacy and quality of medicines circulating on their markets. The problems of ineffective regulation have global implications [8] and minimum requirements for effective medicines regulation should exist in any country to counter poor quality medicines [9].

The situation is severe in sub-Saharan African countries where there is limited resources and pharmaceutical manufacturing capacity with a high disease burden. Thus, parallel, unregulated medicines markets, are posing serious risks for individual and public health [10]. As far as Ethiopia is concerned, there is no comprehensive evaluation of the basic medicines regulatory framework and associated unregulated medicines and their sources.

Ethiopia is one of the sub-Saharan African countries where the pharmaceutical sector is being guided by a national medicine policy [11]. “The Pharmacists and Druggists Proclamation No 43/1942” was the basis for pharmaceutical regulation where both pharmacists and druggists together with the facilities where they practiced were regulated. Comprehensive regulation of the pharmaceutical sector was started in the early stages by a regulation called “Pharmacy Regulation No. 288/1964”, which formed the legal basis for official establishment of drug regulation in the history of the Ethiopia. Pharmacy and laboratory department under the then Ministry of Health was responsible for medicines regulation until June 1999 when a new regulation called the “Drug Administration and Control Proclamation No. 176/1999” was promulgated on 29 June 1999. Following this proclamation, the regulatory component of pharmacy department was transformed to an independent Drug Administration and Control Authority (DACA) of Ethiopia in September 2001 [12]. DACA was restructured as Food, Medicine and Health Care Administration and Control Authority (EFMHACA) of Ethiopia by the “Proclamation No. 661/2009” in 2010 bearing additional responsibilities like regulation of food, health care personnel and settings [13].

The rapid growth and development of pharmaceutical sector after the downfall of the Dergue regime in Ethiopia has led to majority of pharmaceuticals and medical supplies being provided by both the public and private sectors. Currently there are 32 plants (small and large scale) involved in the
manufacturing of pharmaceuticals and related products of which only 12 are manufacturers of generic finished pharmaceutical dosage forms and the remaining are involved in the small scale manufacturing of medical devices, supplies, laboratory reagents, cosmetics, and disinfectants [14]. According to EFMHACA website (www.efmhaca.gov.et), there are 133 importers, 272 wholesalers, 377 pharmacies, 1699 drug shops and 1392 rural drug vendors currently existing in Ethiopia. Some primary data sources reveal that poor quality pharmaceutical products are in the market, because of in-efficiencies in pharmaceutical regulatory functions in Ethiopia [15]. In the past few years, more than 60% of foreign manufacturers have failed to comply with GMP and hence marketing authorization [16]. In the area of post registration testing, low income countries tended to collect fewer samples and report higher rates of products failing testing [17]. For example, the result of trend analysis on quality control laboratory tests carried out in Ethiopia for samples submitted from the year 2007-2011 shows that most of failures of samples submitted for post-marketing surveillance (PMS) was higher (9.5%-15.5%) than samples submitted for the purpose of marketing authorization (4.7% - 10.7%) [16]. Such public health problems should be investigated through critical evaluation of the legal basis and implementation of the pharmaceutical regulatory framework in Ethiopia.

Regular regulatory systems assessment is important for the policy makers in designing or updating policies and strategies to prevent public health from medicines whose safety, efficacy and quality are not ensured and authorized to circulate in the market. The legal basis of the existing pharmaceutical regulatory system in Ethiopia was critically reviewed in comparison with relatively good regulatory systems of three African countries (South Africa, Tanzania and Uganda) and the current EU regulatory system. The comprehensiveness of the legislation to protect the public health was critically evaluated and its practical implementation was assessed through institution-based cross-sectional survey.

2. METHODS

The study was conducted to critically assess the legal framework of the pharmaceutical regulatory system based on Proclamation No.661/2009 and its implementation status on the institutions regulated under the national medicine regulatory authority of Ethiopia. An archival review, in-depth interviews (with key informants selected from institutions involved in the pharmaceutical sector) and institution-based cross-sectional survey using semi-structured questionnaires developed based on WHO guideline were used to gather data [18, 19]. A critical review on the drug regulation was undertaken followed by semi-structured interviews with key
informants from academia, industry and EFMHACA to supplement information gathered from the legal sources.

**Record/archival review**

For the review process, archival review guide was used as a data collection tool. The tool was developed based on WHO guideline [20] and contained detail description on the general content of the medicine legislation and a checklist for the functions of the medicine regulatory authority as evaluation points. The basic purpose for record/archival review was to assess comprehensiveness of the legal framework to protect public health in comparison to medicine regulatory authorities (MRAs) of three selected African countries (South Africa, Tanzania and Uganda) and EU’s European Medicines Agency (EMA). According to a study conducted by WHO in 2006, South Africa had a “fully functional” MRA, whereas Uganda had a “functional” MRA. The study further reported that the MRA of Tanzania and Ethiopia had “potential” [21]. Therefore, selection of the three African countries was based on this literature. EU was selected for its strict medicine regulatory system. The review process further assessed the availability of basic resources to implement the medicines law in relation to marketing authorization/regulatory approvals and regulatory inspections and enforcement in Ethiopia. Overall, records on legal framework; resource for implementation and implementation reports in protecting public health were assessed. Financial and human resource documents; registry for regulatory approval/marketing authorization of medicines, breaches of the law related to unauthorized medicines and their sources, and the associated regulatory measures taken on violations in comparison to penalties provided in the legislation and guidelines were the other focus areas in the record review. Moreover, references on quality of products from the national market were included.

**In-depth interview**

The purpose of in-depth interview was to get details and new insights from the horse’s mouth. A total of 12 key informants selected from different institutions in Ethiopia (EFMHACA, custom authority, Ethiopian pharmaceutical association, Ethiopian druggist association, Ethiopian pharmaceuticals manufacturers association and academia) were interviewed. In-depth interviews were conducted with individuals selected based on their involvement in the regulatory system, and/or role as a representative of an industry or stakeholder group. Interviewees were based within different institutions and from various disciplines: pharmacy, law, chemistry, food technology and management. Several of the individuals interviewed were expert participants with work experience of more than 20 years while the minimum work experience was 10 years. Points of interview
included: (1) the problems related to safety, efficacy and quality pharmaceuticals in the market; (2) government’s political commitment at different levels; (3) adequacy and comprehensiveness of the current legal provision in addressing all the pharmaceutical regulatory activities; (4) the capacity and organization of the regulatory system at various levels in terms of resources and regulatory infrastructures; (5) cooperation and collaboration between these medicine regulatory bodies at different level and their collaboration with other law enforcing agencies such as custom, police and judiciary bodies; and (6) the public support for medicine regulation particularly in reporting illegal medicines circulation.

**Cross-sectional study**

Institutional-based cross-sectional survey was conducted to assess the implementation of those regulatory functions described in the legislation. Semi-structured questionnaires developed based on WHO guideline containing personal information, details of general information on medicines regulation, and unauthorized sources and/or products if any was used to collect data. The institutions-based cross-sectional study was conducted during March to April 2013 to assess the existing regulatory system and its implementation status in Ethiopia. The source population consisted of all pharmacy professionals working in EFHMACA and institutions regulated by EFHMACA. Selected pharmacy professionals from EFHMACA with work experience of two years and above as well as technical managers and marketing personnel of the regulated institutions were included in the study. There were 346 institutions regulated by EFHMACA, from which a sampling frame of 30% (n = 105) was randomly selected for the study. From each of the 105 institutions, two study participants were purposively selected. Out of a total of 84 pharmacy professionals in EFHMACA, 52 participants were included. One hundred ninety seven technical and marketing managers participated in the study, making the total number 249.

**Data collection tools**

Archival review guide, self-administered questionnaires and key informant interviews guide were used as data collection tools.

**Data analysis**

Quantitative data were edited, coded, and analyzed using SPSS Statistics version 16.0 software. Descriptive analyses were conducted and outputs were presented using frequency tables and charts. The data obtained from key informants’ interview were summarized, analyzed and presented in a
descriptive way per thematic area. Similarly, summaries were made from the archival review findings on the critical features of medicine regulation. A multivariable logistic regression was used to investigate the relationship between the different reported factors for the existence of illegal pharmaceutical business.

3. RESULTS

The legal basis of pharmaceutical regulation in Ethiopia: archival review

Legal framework
An overview of medicines regulatory framework in Ethiopia, Tanzania, Uganda, South Africa, and EU is presented in Table 1; while the legal framework of the respective medicine regulatory authorities is presented in Table 2. For all the countries, there exists a well-defined law for medicine regulation with clearly articulated objectives of protecting public health from unsafe, inefficacious and poor quality medicines. Medicine legislation of Ethiopia is based on proclamation 661/2009 with the objective of safeguarding and protecting the public health through ensuring that all medicines (produced locally or imported) that circulate in the market and used in the country are safe, effective, and consistently meet the acceptable quality standards.

In Ethiopia, Parliament approves Proclamations, while regulations are approved by council of ministers similar to that of Tanzania, Uganda and South Africa. In EU, legislations and directives are all approved jointly by European parliament and the council of EU.

Governing body
A good medicines law creates administrative governing bodies to put rules in to practice [22]. In all the studied countries (Ethiopia, South Africa, Tanzania, Uganda and EU), medicine legislations ensure legal provisions for the establishment of a governing body called MRA responsible for enforcing the legislation. EFMHACA is empowered by council of ministers legislation 189/2010 as the governing body for medicine regulation in Ethiopia with its organizational structure presented in Figure 1. Tanzanian Food and Drugs Administration (TFDA), National Drug Authority (NDA), and Medicine Control Council (MCC) are the executive organs to enforce medicines law in Tanzania, Uganda and South Africa, respectively. European Medicines Agency (EMA) is responsible for enforcing medicines law in EU.
Table 1. An overview of the medicine regulatory framework in the selected countries.

<table>
<thead>
<tr>
<th>Features</th>
<th>Ethiopia</th>
<th>Tanzania</th>
<th>Uganda</th>
<th>South Africa</th>
<th>EU</th>
</tr>
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<tbody>
<tr>
<td>Objectives of the current law/proclamation/regulation</td>
<td>Protect public health from unsafe, ineffecti</td>
<td>To ensure that only safe, quality and</td>
<td>To ensure the availability at all times of essential, efficacious and cost-effective drugs to the entire population of Uganda</td>
<td>To provide for the registration of medicines and related substances intended for human and animal use</td>
<td>Protection of public health on the basis of scientific criteria of quality, safety and efficacy of medicinal products concerned.</td>
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<td></td>
<td>cious and poor quality medicine</td>
<td>efficacious products are approved for use in the country.</td>
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<td>Provision/Approvals</td>
<td>Proclamation/Act</td>
<td>Parliament</td>
<td>Parliament</td>
<td>Parliament</td>
<td>European parliament/the council of EU</td>
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<td>Ministry upon advise of TFDA</td>
<td>Ministry upon advise of NDA</td>
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<td></td>
<td>EFMHACA</td>
<td>TFDA</td>
<td>NDA</td>
<td>MCC</td>
<td>EMA</td>
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<td>Negarit Gazette</td>
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<td>Gazette</td>
<td>Gazette</td>
<td>Official Journal for EU L 136</td>
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<tr>
<td></td>
<td>EFMHACA and RRBs</td>
<td>TFDA</td>
<td>NDA</td>
<td>MCC</td>
<td>EMA, EDQM, national competent authorities</td>
</tr>
</tbody>
</table>

EFMHACA: Food, medicine and healthcare administration and control authority of Ethiopia; TFDA: Tanzania food and drug administration; NDA: National drug authority of Uganda; MCC: Medicine control council of South Africa.

The overall responsibility and accountability for all aspects of medicine regulation is given to a single agency in Tanzania, Uganda, South Africa and EU, while it is distributed horizontally between EFMHACA and Ministry of agriculture and rural development, and vertically between EFMHACA and regional state regulatory bodies (RRBs) in the case of Ethiopia. Ministry of agriculture and rural development is responsible to regulate veterinary medicinal products, where as RRBs are responsible to regulate establishments in the distribution chain except importers and wholesalers.
### Table 2. The legal framework of the medicine regulatory authorities (MRAs).

<table>
<thead>
<tr>
<th></th>
<th>Ethiopia</th>
<th>Tanzania</th>
<th>Uganda</th>
<th>South Africa</th>
<th>EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legal status of the regulatory authority</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Main regulatory authority</td>
<td>EFMHACA</td>
<td>TFDA</td>
<td>NDA</td>
<td>MCC</td>
<td>EMA</td>
</tr>
<tr>
<td>Supervisory body</td>
<td>MOH and RHB</td>
<td>MOH</td>
<td>MOH</td>
<td>MOH</td>
<td>MOH</td>
</tr>
<tr>
<td>Links with other local regulatory agency</td>
<td>MOARD &amp; RRB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Member states</td>
</tr>
<tr>
<td>Line of command</td>
<td>Multiple agencies</td>
<td>Single agency</td>
<td>Single agency</td>
<td>Single agency</td>
<td>Single agency</td>
</tr>
<tr>
<td>Power to hire or fire personnel</td>
<td>Yes*</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Financial independence</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

A = semi-autonomous statutory authority under Ministry of Health (MOH), B = council under MOH, NA = not applicable, RHB= Regional states Health Bureau, * available at federal level

**Figure 1.** Organizational structure of Food, Medicine and Health care Administration and Control Authority of Ethiopia.
Regulatory provisions to MRAs
The content and domains of regulations of Ethiopia, Tanzania, Uganda, South Africa and EU is presented in Table 3. In terms of its content and domain of regulation, proclamation 661/2009 of Ethiopia covers most of the critical features for medicine regulation with provisions for the major regulatory processes. Pharmaceuticals are subject to numerous controls at all levels, and EFMHACA is granted to regulate their manufacture, distribution, marketing, prescribing, labeling and dispensing. However, there were some differences between legal provisions in Ethiopia and the other countries whose legislations were reviewed for comparison. The variations lie on the domains of regulation like scope of regulated products (e.g. veterinary medicines are excluded from EFMHACA), price control and overall responsibility distribution for medicines regulation.

Product classification
Pharmaceutical legislations provide product classifications based on definitions for medicines or medicinal products. Product classification is important for executing pharmaceutical laws governing medicines production, marketing and utilizations.

According to Article 1 of Directive 2001/83/EC of EU, a medicinal product is defined as: (a) any substance or combination of substances presented as having properties for treating or preventing disease in human beings/animals; and/or (b) any substance or combination of substances which may be used in, or administered to, human beings, either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis. Moreover, Article 2(2) of the Directive provides classification of a product into a medicinal product, where doubt remains as to its classification as a medicine or another type of product; but the European Court of Justice judgment is helpful. Product classification in EU considers presentation and/or purpose/function aspects of the products.

Article 2(6) of Proclamation No. 661/2009 of Ethiopia defines medicine as any substance or mixture of substances used in the diagnosis, treatment, mitigation or prevention of a disease in human and includes narcotic drugs, psychotropic substances and precursor chemicals, traditional medicines, complementary or alternative medicine; poisons, blood and blood products, vaccine, radioactive pharmaceuticals, cosmetics and sanitary items and medical instruments.

Product classification in Ethiopia (and the three African countries) addresses only the functional issue of products and neglects the presentation aspects, which are well described in EU. It poses greater challenges to classify products that are on the borderline between medicinal products and food supplements, biocides, cosmetic products and medical devices. Moreover, advanced therapy medicinal products (ATMP) like gene therapy, somatic cell therapy and tissue engineering are not
classified in Ethiopia and in either of Tanzania, Uganda and South Africa. Therefore, EU’s product classification is much broader and provides a wider scope of products to be regulated under the law.

Table 3. Content of medicine laws and some regulatory activities of Ethiopia, the three African countries and EU.

<table>
<thead>
<tr>
<th>Product classification</th>
<th>Ethiopia</th>
<th>Tanzania</th>
<th>Uganda</th>
<th>South Africa</th>
<th>EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human medicine</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Veterinary medicine</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Medical devices</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Blood &amp; blood products</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Tissue transplant</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Cosmetics</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Narcotics &amp; psychotropic</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Herbal products</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Functional foods</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Food and additives</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>ATMP</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Border-line products</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>●</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Regulatory functions</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Product assessment and registration</td>
<td>Human medicine</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Veterinary medicine</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Herbal products</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Biological products</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Cosmetics</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Foods and additives</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Premise licensing</td>
<td>Manufacture</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>importers/wholesalers</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>retail outlets</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Inspection</td>
<td>GMP inspection</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Supply chain inspection</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>Quality control</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>Pharmacovigilance</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Control of product promotion</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Clinical trial control</td>
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<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>Import/export control</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Price control</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Sources of products regulated</td>
<td>Private manufacture</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Government manufacture</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Private import</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Government import</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Control of raw materials</td>
<td>Active pharmaceutical ingredient (API)</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>Excipients</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Registration harmonization</td>
<td></td>
<td>EAC and SADC</td>
<td>EAC</td>
<td>EAC and SADC</td>
<td>ICH</td>
</tr>
</tbody>
</table>

*Present; ○Absent; EAC: East African community; SADC: South African development communities; ICH: International conference on harmonization; 'not under FMHACA; "Except importer/wholesalers, lower supply chains are regulated by regional state regulatory bodies in Ethiopia; ATMP: Advanced therapy medicinal products; Border-line products: between medicinal products and food supplements, biocides, cosmetic products or medical devices.*
Product assessment and registration

Medicines registration, also called marketing authorization, is often a major element in national pharmaceutical law. It is carried out by MRAs to ensure that a medicinal product has been adequately tested and evaluated for safety, efficacy and quality and the product information provided by the manufacturer is accurate.

The comparative presentation of market authorization by EFMHACA, TFDA, NDA, MCC and EMA is given in Table 4. All the MRAs have a legal basis for marketing authorization of the pharmaceutical products with guidance for applicants and standard operating procedures for assessors. Except EFMHACA, all the MRAs make use of external experts in the form of various committees and are involved in regional or international harmonization of registration process. EFMHACA has a single advisory committee, and is not currently participating in regional harmonization for registration, even though Ethiopia is very recently working towards harmonization of quality assurance for pharmaceutical and medical products with Intergovernmental Authority on Development (IGAD) member countries.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Countries</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Legal basis for authorization</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Guidance for applicants</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>SOP for assessment</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Advisory committee(s)</td>
<td>□</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Assessors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>● External Assessors</td>
<td>○</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>● Full time assessors</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Legal provision to publish list of approved products</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Recognition of other MRA decision</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Harmonization of registration</td>
<td>○</td>
<td>EAC, SADC</td>
<td>EAC</td>
<td>SADC</td>
</tr>
<tr>
<td>Fast track registration</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Collect fee for application</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Target time frame to assess (in Months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>● New medicines</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>● Generic medicines</td>
<td>6</td>
<td>4.5</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>● Fast track applications</td>
<td>6</td>
<td>1.5</td>
<td>6</td>
<td>●</td>
</tr>
<tr>
<td>Registration validity period (years)</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

S: Stringent MRA and recognized by WHO prequalification program; ●: Yes; ○: No; □: Yes but not functional during the assessment; SADC: Southern African Development Communities; EAC: East African Community; ICH: International Conference on Harmonization.

Article 20 of Regulation 299/2013 of Ethiopia grants special permit for importation of unregistered medicinal products for clinical trials, scientific investigations, personalized use of a patient, laboratory quality testing for the purpose of registration, disaster and emergency aids, diplomatic missions and
treatment of diseases with no adequate attention. Even though very limited, EU also provides exceptions for medicinal products for clinical trials, emergency situation and compassionate use.

Similar to other MRAs, the market authorization process in Ethiopia includes manufacturing premise inspection for GMP compliance, assessment of product dossiers and laboratory testing, where applicable. The requirements for Ethiopian market authorization are: (1) the medicinal product has to be included in to national medicine list; (2) the manufacturing site has to be approved and certified for compliance with GMP either by EFMHACA or other recognized stringent regulatory authorities and (3) such GMP certified or waived manufacturers have to submit application for dossier evaluation and product quality assessment accompanied with application fee.

**Licensing and inspection**

Law should create mechanisms to ensure that relevant parties are licensed and inspected so that the community can have confidence in them [21]. Proclamation 661/2009 states that involvement of any person or institution in the pharmaceutical sector without being authorized or licensed is legally prohibited. A breach of law to trade medicine without certificate of competence shall be punishable with imprisonment of 5-7 years and fine of 2,700-5,400 USD [15].

Medicines retail outlets and supply chain inspections are mandated to RRBs, while EFMHACA is responsible for manufacturers, importers and wholesales inspection and licensing unlike TFDA, NDA, MCC and EMA. Moreover, the EFMHACA proclamation does not include requirements for obtaining licenses, terms and conditions for suspending or revoking activity and product licenses. It does not define the norms, standards and specifications to be applied in assessing the quality, safety and efficacy of medicinal products. These points and other similar details were left to be stated in the directives and guidelines to be prepared by EFMHACA based on the proclamation.

A review of 2010-2012 performance and activity reports indicated that EFMHACA is striving to exercise the legal mandate through preparations and approvals of different directives, standards, guidelines, standard operating procedures and check lists. Accordingly, the authority has prepared 39 standards, 14 directives and various guidelines in the last three years since proclamation 661/2009 has been enacted.

**Control of raw materials**

Proper quality management of pharmaceutical raw materials and excipients during collection, import, export, transport, distribution, storage, processing, and documentation is a base to obtain safe, efficacious and good quality pharmaceutical products. Therefore, the source, origin and suitability of the starting material should be clearly defined and controlled.
In all the assessed MRAs, the finished pharmaceutical product (FPP) manufacturers are responsible for the control of raw materials (active pharmaceutical ingredients (APIs) and excipients). For EU, all imported active substances must have been manufactured in compliance with standards of good manufacturing practices (GMP) at least equivalent to the GMP of the EU [23], where as for Ethiopia, compliance with Pharmacopoeia specifications and GMP procedures is adequate.

**Implementation of Medicines regulation in Ethiopia**

To address the implementation status of medicines regulatory system in Ethiopia, the results of in-depth interview of key informants and institutions-based cross-sectional survey were used.

**Key informants perspective**

All the key informants agreed that proclamation 661/2009 is comprehensive in addressing all the pharmaceutical regulatory activities to protect public health. However, the key informants stressed that the important regulatory tools that enable implementation of the proclamation have to be put in action since the sole existence of the law does not ensure its implementation.

**Government’s political commitment**

All the key informants believe that there is strong political commitment from the government to support the pharmaceutical sector in general and the regulatory system in particular. The government has already denoted the regulatory system as one of the pillars in the health sector. It produced provisions of proclamation for regulation of the pharmaceutical sector, empowered the regulatory authority to hire staff and acquire resources, established procurement system to supply government health institutions with pharmaceuticals and planned to enhance local production in its growth and transformation plan (GTP). The GTP capacitates local manufacturers and attracts investors to the pharmaceutical sector. However, the informants emphasized that more should be done to establish a strong medicine regulatory. According to them, the physical existence of the law, unless supported with a proper organization, qualified human resource and adequate finances, is not a guarantee for effective medicines regulation.

**Product smuggling**

According to the key informants, pharmaceutical products smuggling is recently emerging in Ethiopia and much worsening in remote areas of the country due to weak custom control. Some even claimed that they have had observed similar problems even in the central part of the country including Addis Ababa.
The key informants mentioned that legally imported products were also diverted to the private sector. Some added that the problem was not only smuggling, but also the condition in which such products were transported and stored. The smuggled products are usually transported using animals through deserts without any care for the storage conditions, which could cause further damage or degradation to these products.

The majority of the key informants believed that both professionals and non-professionals in the legal and illegal institutions were stakeholders and responsible for the problems. Although some importers and distributors may be involved, the primary destinations of such illegal products were retail outlets and unregulated markets from which they were dispensed to the end users. Some of the key informants claimed that clinics were providing diagnostic and pharmaceutical services together for which they were not licensed.

As per the key informants, the main factors contributing to the existence of illegal products in the market were weak regulatory enforcement (64.5%), poor inter-agency cooperation between law enforcing bodies (62.2%), and weak boarder control (50%). Decentralization of the regulatory activities to lower-level administrations with weak control capabilities created regulatory gaps and contributed for smuggling or diversion of the pharmaceutical products in Ethiopia.

**Harmonization with-in law enforcing agencies at various administrative levels**

Efforts of cooperation have been undertaken between medicine regulatory bodies at federal, regional states and other law enforcing agencies according to the EFMHACA informants. However, there is no established system with clearly defined roles and responsibilities of parties involved including inter-agency standard operating procedures. The informants added that in particular there is weak cooperation between the authority and the prosecutors at court, and thus most illegal cases taken to court were not successful.

**Cross-sectional survey**

Data were collected from a total of 249 respondents using self administered questionnaire with the response rate of 94.3%. The majority of the institutions, 175 (71.1%) were from the central part of the country, *i.e.* Addis Ababa and its surrounding, since the majority of the institutions were concentrated around the capital.

**Awareness about medicine regulation in Ethiopia**

Of the total 249 respondents, 197 (79.1%) were not staff of EFMHACA, of which only 83 (42.1%) reported awareness about the current medicine legislation of Ethiopia. The majority, 67(80.7%), of
those who have had awareness, reported that the medicine legislation is comprehensive enough to cover important pharmaceutical regulatory activities to protect public health.

**Resources for medicine regulation**

The study participants were EFMHACA staff members. They revealed that there is significant shortage of qualified and skilled human resource for medicine regulation in Ethiopia. Low salary, lack of attractive carrier structure and incentives were reported to be the leading contributing factors to hire and retain qualified and skilled personnel within the regulatory system. Regarding the financial resources, the study participants confirmed that there was no adequate financing to perform the routine regulatory activities due to insufficient government funding and weak revenue generating system from services, even though EFMHACA is mandated to use the revenue it generates from service delivery.

**Unauthorized medicines sources**

Out of the total respondents, 102 (41.0%) reported that there were institutions and/or individuals involved in pharmaceutical business without being authorized or licensed to provide such services. According to the study participants, these unauthorized/illegal institutions are involved in importation and distribution to the medicine retail outlets (pharmacies, drug shops, rural drug venders, clinics), and even dispensing directly to the users. The study revealed that there were unauthorized sources for pharmaceutical products in all the major commercial cities of the country; with majority in the eastern part (71.4%, 10 of 14) followed by the northern region (53.6%, 15 of 28) of the country.

**Illegal pharmaceutical products**

Seventy eight (31.3%) respondents reported that illegal pharmaceutical products are circulating in the pharmaceutical market of the country in the last 12 months preceding the study. It was reported that illegal pharmaceutical products enter into the distribution channel either through legal or illegal ports. However, the majority of the respondents believed that these products enter through illegal entry routes as presented in Figure 2.
The majority of the study participants (158, 63.5%) reported that the main contributing factor for the presence of illegal sources was weak law enforcement. All the reported factors are presented in Figure 3.

It was reported that legally imported pharmaceutical products are diverted from public to private sectors. Anti-infective medicines (50%) were the most frequently reported illegal pharmaceutical products (from which anti-malarial medicines cover more than a third) followed by hormonal drugs (insulin and oral anti diabetics, and sex hormone preparations) and contraceptives (21%).

In a binary logistic regression analysis, variables such as, inadequate law enforcement/regulatory measures on illegal institutions, lack of informal market control, poor control at entry ports, poor cooperation between FMHACA and regions, availability of illegal medicines, extra profit from illegal products, and reporting illegal product were found to have significantly association (p < 0.05 at 95% CI) with the existence of illegal institutions in the pharmaceutical sector of the country (Table 5).

From the multivariate logistic regression analysis performed on these variables, it was found that inadequate regulatory measures/enforcement, lack of informal market control, availability of illegal pharmaceutical products and location (site) of the country from the entry ports were remained significantly associated with the existence of illegal institutions.

Inadequate regulatory measures/enforcement on violations was found to be strong contributing factor for the existence of unauthorized sources. Illegal institutions or individuals are more than three times significantly likely to exist in the pharmaceutical market when regulatory measures/Enforcements are inadequate than when such measures are adequate (AOR = 3.5, 95% CI = (1.84,
6.65), at p < 0.001). Similarly, illegal institution are two times more likely to exist in the pharmaceutical market when informal market is unregulated than when it is regulated (AOR = 1.97, 95% CI = (1.08, 3.595 at p < 0.05). The illegal sources are three times significantly more likely to exist in the presence illegal pharmaceutical products circulating in the market (AOR = 2.785, 95% CI for OR (1.52, 5.11), at p ≤ 0.001).

![Figure 3](image-url)

**Figure 3.** Factors contributing to the availability of illegal pharmaceutical sources (n = 245), Ethiopia, March 2013.

**Table 5.** Selected factors on existence of illegal pharmaceutical institutions and products, Ethiopia, March 2013.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Existence of illegal institutions</th>
<th>Crude OR (95%CI)</th>
<th>Adjusted OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>p-value</td>
</tr>
<tr>
<td>Inadequate regulatory measures</td>
<td>Yes</td>
<td>84(53.2%)</td>
<td>74(46.8%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>18(20.5%)</td>
<td>70(79.5%)</td>
</tr>
<tr>
<td>Port control</td>
<td>Yes</td>
<td>42(34.1%)</td>
<td>81(65.9%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>60(48.8%)</td>
<td>63(51.2%)</td>
</tr>
<tr>
<td>Lack of informal market control</td>
<td>Yes</td>
<td>74(49.7%)</td>
<td>75(50.3%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>28(28.9%)</td>
<td>69(71.1%)</td>
</tr>
<tr>
<td>Lack cooperation b/n FMHACA &amp; regions</td>
<td>Yes</td>
<td>50(33.6%)</td>
<td>99(66.4%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>52(35.6%)</td>
<td>45(64.4%)</td>
</tr>
<tr>
<td>Illegal product exist</td>
<td>Yes</td>
<td>46(59%)</td>
<td>32(41%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>56(33.3%)</td>
<td>112(66.7%)</td>
</tr>
<tr>
<td>Reporting illegal product</td>
<td>Yes</td>
<td>20(60.4%)</td>
<td>13(39.4%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>82(38.5%)</td>
<td>131(61.5%)</td>
</tr>
<tr>
<td>Extra profit from illegal product</td>
<td>Yes</td>
<td>50(49%)</td>
<td>52(51%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>52(36.1%)</td>
<td>92(63.9%)</td>
</tr>
</tbody>
</table>
The majority of the respondents claimed that they did not usually report the presence of illegal pharmaceutical institutions (53.9%, 55/102) and products (59.0%, 46/78). Details on awareness and reporting practice of the respondents is presented in Table 6. The main reasons for not reporting the problems were lack of formal reporting system, fear of security problems from smugglers, absence of legal measures for previous reports and lack of awareness to whom to report.

Table 6. Awareness and reporting practice of respondents on problems related to pharmaceutical products in the market, Ethiopia, March 2013.

<table>
<thead>
<tr>
<th>Problems related to pharmaceutical product</th>
<th>Aware of problem</th>
<th>Reported problem Frequency (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illegal institutions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>102 (41.5%)</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>144 (58.5%)</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>Total</td>
</tr>
<tr>
<td>Illegal products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>78 (31.7%)</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>168 (68.3%)</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>Total</td>
</tr>
<tr>
<td>Safety problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>98 (39.8%)</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>148 (60.2%)</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>Total</td>
</tr>
<tr>
<td>Drug abusers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>38 (17%)</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>186 (83%)</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>Total</td>
</tr>
<tr>
<td>Misleading/in-accurate medicine promotion materials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>43 (17.5%)</td>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
<td>203 (82.5%)</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>246</td>
<td>Total</td>
</tr>
</tbody>
</table>

*Percentage calculated of total aware or ‘yes’ at each point not of total respondents which is 246

4. DISCUSSION

An effective national pharmaceutical law is a primary means of ensuring that pharmaceutical policy goals are achieved while the unique character of pharmaceutical products, personnel and facilities is preserved. Therefore, when assessing the pharmaceutical regulatory system in Ethiopia from the legislation point of view, in terms of its purpose and content, comparative review had shown that the medicine legislation in Ethiopia provides basic legal framework and covers all products for which medicinal claims are made, as well as related pharmaceutical activities, in both the public and private sectors. In line with this, the cross-sectional study revealed that the legislation is comprehensive enough to cover all pharmaceutical regulatory activities important to protect public health.

Considering the significant public health implications of veterinary medicines in human health, the legislation in Ethiopia does not have provisions for the control of veterinary medicines in contrast to all other countries with which the comparative review was conducted. However, Ethiopia has a separate authority called veterinary drugs and animal feed administration and control authority
established by Proclamation No. 728/2011 to regulate the proper production, distribution and use of veterinary drugs to ensure safety, efficacy and quality of the products and to enhance the productivity and health of the livestock population. In fact, there should be at least exchange of information between this authority and EFMHACA with regard to full regulation of products, premises and practicing personnel.

The Ethiopian legislation mandated EFMHACA to prepare and approve or submit to appropriate organ for approval of regulatory standards and specifications to be applied in assessing the quality, safety and efficacy of medicinal products and upon approval to ensure the implementation. Accordingly, a number of guidelines including guidelines for human medicine evaluation, registration, and premises licensing and inspection have been prepared by EFMHACA got approval. However, the problem with such mandate is that, guidelines approved by the regulatory authority are only administrative tools rather than statutory instrument in the court unless approved by council of ministers; which in turn has an influence on enforcement of the law. Moreover, there were no reports found on joint operations between EFMHACA and other law enforcing agencies such as police and custom authority and this could be taken as additional evidence for lack of inter-agency cooperation in Ethiopia, which are very critical for effective medicine regulation [5, 8]. Such operational cooperation was reported in other African countries and the experience in Uganda revealed a success story. A number of unregistered drug outlets were uncovered and many counterfeit products were identified during joint operations between law enforcing agencies (MRA, custom, and police) in the span of 2007-2011 in Uganda [24]. The other major finding was that the responsibilities for pharmaceutical regulation in Ethiopia are distributed horizontally between two ministries; and vertically between federal and regional state governments. Such fragmented systems among agencies could lead to overlap of responsibilities and regulatory ineffectiveness [24, 25] resulting in wastage of resources in the already poor-economy [26].

Literatures recommend harmonized optimal drug registration approach for resource-limited settings, which should reliably evaluate safety, efficacy, and quality of drugs for use [27-29]. However, Proclamation 661/2009 of Ethiopia does not have any article on regulatory harmonization with respect to market authorization unlike that of Tanzania, Uganda, South Africa and EU.

Even though medicine legislation in Ethiopia prohibits the involvement in medicine trade without being licensed and sales by licensed importer and wholesaler to person or institution without certificate of competence, there exist institutions and/or individuals involved in pharmaceutical business without being certified to provide such services. Illegal transits and distributions are usually secret for regulatory inspections by custom or medicine regulatory bodies [30], and their existence could not only indicate inefficiencies in regulating the sector but also the presence of either
unauthorized sources of medicine to the country or pharmaceuticals diversions. Therefore, it is reasonable to assume that these unlicensed institutions were the destinations for smuggled or diverted pharmaceutical products. This is in line with the reports of the study participants that such illegal institution or individuals are in an informal market for medicines and similar finding reported the existence of non-conventional market for pharmaceutical products in Ethiopia [31], which could lead to the high prevalence of poor quality medicines.

The study participants reported that illegal medicines included both registered and unregistered products of which anti-infective medicines take the major share. Among those medicines imported illegally, it is obvious that some were counterfeits and/or substandard. A national quality survey study conducted in Ethiopia reported a significant proportion of poor quality albendazole, mebendazole and tinidazole tablets on the Ethiopian market from which 29% was substandard [32]. This study reported diversion of medicines. Another study conducted on assessment of anti-malarial diversion in 11 African cities including Addis Ababa showed that 6.5% (58 of 894) samples of anti-malarial medicines collected from market were found to be diverted across at least one national border as determined by visual inspection [33]. Diversion of medicine primarily affects sustainable procurement systems, because it exacerbates stock outs in public health sector exposing patients to extra costs and making criminals profitable from diversion.

Inadequate enforcement of law was found to be the leading contributing factor for the presence of illegal institutions and/or products. Similarly, literatures revealed that inadequate legislation and weak or insufficient law enforcement along with resource constraints to effectively implement the law are among the regulatory factors that have contributed to the illegal circulation of medicines in many countries [34]. Experiences in Australia, Canada, and the United States have shown that adequate legislation and its enforcement result in fewer poor-quality medicines and greater public confidence in the quality of the medicines [30]. Although the evidence for the feasibility of strict regulatory enforcements is very limited in low income countries, such interventions to improve regulatory compliance was reported to have impact on illegal sources in Vietnam and Lao Peoples Democratic Republic [35].

The strength of the study is that it included the strict EU regulatory framework and that of three African MRAs to evaluate the legal basis of Ethiopian pharmaceutical regulation. Moreover, it tried to address both the regulator and the regulated firms and professionals in the pharmaceutical sector. The other strength was the geographic coverage of the study, which was wider and encompassed the major trade cities and parts of the country.

Most archival data are collected for nonscientific reasons and thus often do not suit the purpose of the researcher. Moreover, pharmaceutical sector regulation is so sensitive and obtaining reliable and genuine data could be difficult.
5. CONCLUSIONS

The study revealed that medicines regulation as a system in place in Ethiopia has potential capacity to develop in comparison with EU; and the three African countries like South Africa, which was reported by WHO in 2006 to have fully functional MRA. But this study revealed that unauthorized/illegal medicine sources exist in the sector due to some in-efficiencies in the implementation of this regulatory system into the real practice. For instance, resource constraints as a basic factor along with other reported factors such as lack of informal market regulation, weak enforcement of the law and availability of illegal products are regulatory factors. Less reporting practice of illegal activities by professionals in the sector was also another important factor associated with the existence of illegal institutions in the pharmaceutical market.

The majority of reported medicines as illegally imported were anti-infective medicines including anti-malarial and other antibiotics. These reported products included both registered and unregistered products. Unregistered products do not only escape the necessary government tax but they are also medicines for which quality, safety and efficacy are not ensured. Hence, they could be counterfeit, substandard and/or degraded products because of their transportation and storage conditions to hide from customs and regulatory authority. In fact, registration is not a guarantee of quality for those medicines which are illegally supplied (may be in bad transportation and/or storage conditions) but which have passed the formal registration process.

Proclamation 661/2009 should be amended to include critical legislative gaps like harmonization. Product classification should be revised so that a strong autonomous regulatory system should take place. RRBs should have mechanisms of cooperating with EFMHACA. EFMHACA should improve awareness about the current legal provision for medicine regulation. Moreover, detail national assessment of pharmaceutical malpractices and poor quality medicines should be conducted.

6. REFERENCES


GENERAL CONCLUSIONS

“If ... the past may be no rule for the future, all experience becomes useless and can give rise to no inferences or conclusions.”

David Hume
(“1711 - †1776, Scotish philosopher and economist)
GENERAL CONCLUSIONS

In Chapter I, we described the general introduction about the thesis with the thesis outline and objectives. As described in Chapter I, quality of pharmaceutical drugs is a topic of global concern and the most important public health aspect. Quality concepts and definitions were clearly described and related to factors that cause poor quality medicines. Quality is built into a medicine during its design, development, and manufacture. Therefore, manufacturers are primarily responsible for the quality of the pharmaceuticals they produce following good manufacturing practices (GMP). After a pharmaceutical product leaves the manufacturer’s premises, distributors, procurement agencies (purchasers), dispensers, and users are responsible for maintaining the quality of the product through proper storage, transport, distribution, dispensing, and use. National medicines regulatory authorities are responsible for ensuring that manufacturers comply with current GMP requirements and execute the key regulatory functions that are required to maintain product safety, efficacy and quality through out the supply chain until the end user; which may present a challenge for countries with limited resources. Therefore, the overall objective of this thesis was described to be to contribute to quality of medicines circulating in resource-limited settings in general and Ethiopia in particular from analytical and regulatory point of view.

In Chapter II, we applied a risk-based QbD approach to establish a drug quality laboratory by the name Jimma University Laboratory of Drug Quality (JuLaDQ) in Jimma, Ethiopia. The establishment of the laboratory considered the various categories of quality risks associated with the expected routine analytical procedures using failure mode effect analysis (FMEA) and devised control measures using quality system model that define the end results, analytical quality with reduced variability. Therefore, the analytical results produced by JuLaDQ are accurate, traceable and reliable and the established laboratory is fit-for-purpose.

In Chapter III, a stability-indicating HPLC-UV method for the determination of lumefantrine in FDC products was developed and validated. Then, an exhaustive impurity profiling of lumefantrine was performed using HPLC-UV/ESI-ion trap MS. From unstressed, stressed and accelerated stability samples of lumefantrine API and FPPs, nine compounds were detected and characterized to be lumefantrine related impurities. One new lumefantrine related compound, DBK, was identified and characterized as a specified degradation impurity of lumefantrine in real market samples (FPPs). The in-silico toxicological investigation (Toxtree® and Derek®) indicated overall a lesser toxicity for the specified impurity DBK compared to the API lumefantrine itself. Moreover, a GC-FID method for
lumefantrine assay in pharmaceutical preparations was also developed and validated within an analytical QbD approach. The method is linear, precise and sensitive. It makes use of simple sample preparation procedures and is not solvent consuming. The retention time of lumefantrine was 26.0 min and there was no interference from its related synthesis and degradation impurities and excipients. The developed method was successfully applied to analyze lumefantrine content in different marketed anti-malarial FPPs and can thus be applied to routine quality control of lumefantrine in pharmaceutical preparations.

In Chapter IV, a stability-indicating HPLC method for simultaneous assay of β-artemether and lumefantrine fixed dose combination anti-malarial products was developed, using a fused-core reversed-phase amide stationary phase combined with an isocratic acetonitrile sodium phosphate mobile phase [Acetonitrile/1 mM phosphate buffer pH 3.0 (52:48, v/v)]. It is a rapid (four minutes total run time), precise and accurate method that can be utilized to quantify these anti-malarials in the presence of their related degradation products or impurities produced during inadequate transportation and storage. This method can be applied in the routine regulatory quality control of β-artemether and lumefantrine containing FDC drug products. The in-silico toxicological investigation using Derek Nexus® indicated overall a toxicity risk for β-artemether-related impurities comparable to that of the API β-artemether itself. Moreover, quality survey of ACT FDC anti-malarial products was conducted in Jimma zone (Oromia regional state), southwest Ethiopia. The study revealed that there were no falsified packages and labels for the samples obtained from formal outlets. All the sampled ACT products did contain the stated active ingredient and all (except one sample with 111% lc for lumefantrine) comply with the acceptance criteria set in Ph. Int. for assay (90-110 % lc) and Ph. Eur. for mass uniformity. Even though this is good for the malaria control program of the country, it does not necessarily imply the efficiency of the overall national regulatory system of Ethiopia, including the private sector as well. Therefore, regulatory and policy strategies including nationwide surveys of quality of medicines for the containment of poor quality anti-malarials should always be active and implemented.

In Chapter V, nationwide quality survey of MEB, ALB and TNZ was conducted in the legal distribution chain of Ethiopia as a reality check. The survey study indicated that all sampled products did contain the stated active ingredient, but poor quality products were identified in all three medicines and collection sites in the country due to non-compliant assays, inadequate drug release of required dose or toxicity concerns due to over-dosage of some of the medicines containing higher level of active ingredient. Over-dose in the assay values of the three studied drugs is not a direct clinical concern related to adverse effects where as under-dosing constituted one of the risk factors for the development of resistance. The study further identified the most critical quality attributes in product quality assessment using FMEA risk-based quality evaluation of the three drugs where assay was
found to be the most critical quality attribute with highest RPN. Moreover, it was revealed that Derringer’s desirability function can be applied to pharmaceutical quality assessment using Psychophysical Harrington’s scale of quality where products could be classified into excellent, good, acceptable, low and bad quality.

In Chapter VI, the pharmaceutical regulatory framework of Ethiopia is critically reviewed from legal basis and implementation point of view. From legislation point of view, medicines regulation as a system in place in Ethiopia has potential capacity to develop in comparison with EU; and the three African countries such as South Africa, which was reported by WHO in 2006 to have fully functional MRA. But this study revealed that unauthorized/illegal medicine sources exist in the sector due to some inefficiencies in the implementation of this regulatory system into the real practice. For instance, resource constraints as a basic factor along with other reported factors such as lack of informal market regulation, weak enforcement of the law and availability of illegal products are regulatory factors. Less reporting practice of illegal activities by professionals in the sector was also another important factor associated with the existence of illegal institutions in the pharmaceutical market. The majority of reported medicines as illegally imported were anti-infectives including antimalarial and other antibiotics. These reported products included both registered and unregistered products. Unregistered products do not only escape the necessary government tax but also they are medicines for which quality, safety and efficacy are not ensured. Hence could be counterfeit and substandard products or degraded products because of their transportation and storage condition to hide from customs and regulatory authority.
GENERAL CONCLUSIONS
BROADER INTERNATIONAL CONTEXT, RELEVANCE AND FUTURE PERSPECTIVES

"Take control of your future by taking a choice of starting it right now."

Auliq Ice
(American singer, songwriter and author)
Sir Winston S. Churchill (1874 – 1965: UK prime minister from 1940 to 1945 and 1951 to 1955) quoted that “Healthy citizens are the greatest assets any country can have”. For the wellbeing of all citizens, access to health services of appropriate quality is critical. According to the world health organization (WHO), access to quality medicines was identified as one of the prerequisites in the universal health coverage; but is often a neglected issue in developing countries. To improve this situation, the overall objective of this PhD dissertation was to contribute to the quality enhancement of medicines in Ethiopia and the whole region of the horn of Africa (HOA).

According to international conference on harmonization (ICH), product quality does not happen by accident, nor is it acceptable under the current regulatory guidelines to use end-product testing to achieve quality by scrapping product that does not meet specifications. Rather, quality must be created into the process and product, and this can be accomplished only when there is extensive scientific knowledge of the desired product characteristics and the processes that transform the incoming materials into the final product. Such quality-by-design (QbD) principles, already used since decades in other industries, have been slowly -but now broadly- applied in the pharmaceutical field for process and product development; as well as in analytical method development. However, its application in setting-up facilities like pharmaceutical quality control (QC) laboratories is new. This new insight of extending the existing product and analytical QbD semantics to laboratory QbD is getting formal recognition from WHO prequalification inspectors as it was explicitly described in their Jimma University Laboratory of Drug Quality (JuLaDQ) inspection (October 2015) result report of November 2015. Utilizing the knowledge, skills and experience obtained from and during this PhD activity, we were able to set-up a pharmaceutical QC laboratory in a resource-limited setting by applying a scientific risk-based QbD approach. JuLaDQ, the fully functional GMP-compliant QC-lab set-up in Jimma/Ethiopia, can contribute to the quality enhancement of medicines both nationally and in the whole HOA region. The capacity developed at this particular facility could serve as a hub to strengthen both national and HOA-regional pharmaceutical quality systems. It can contribute to the supply of quality medicines of direct relevance to priority national and regional health programs by providing QC analytical services through collaboration with both national and international
stakeholders, contributing in countering the problem of poor quality medicines, the enormous global public concern with major prominence in developing countries.

Since the QC-lab is university-based, it also aims at integrating QC-services, training (short- and long-term, and on the job training) and research in the area of advanced pharmaceutical quality-related sciences. These roles will ensure that the lab provides the technical leadership and global advocacy regarding the importance of medicines quality and its required analytics. Through its research and development, improved quality tools and methods for medicines can be developed and disseminated. Moreover, broader and focused long term and high level knowledge and skill based research projects that can accommodate new PhD programs are being developed.

In this PhD dissertation, we developed improved and advanced analytical methods of separation and detection for the selected medicines. The methods were communicated to the international community through publications and symposia presentations: these can thus be evaluated by other laboratories, eventually adapted, and transferred to interested manufacturing companies, regulatory authorities, research organizations and institutions, national and/or international laboratories. The developed methods were technically kept as simple as required to solve the quality question, while also considering the economics of time, solvents and equipment/columns for the lab-users.

In this PhD project, we developed also a new risk-based desirability function approach which can be used in surveys of medicines quality. This approach combined the existing knowledge about the chemical analytics of the medicines of interest with that of the ICH-recommended risk-based quality ranking by incorporating the clinical relevance of quality attributes. Moreover, by combining the individual quality attributes into one global quality number using the desirability function approach, this allows a more quantitative overall quality ranking. This is a new approach in medicines quality assessment, which can be applied in for example the comparative and time-based quality evaluations by national and international authorities.

This PhD project also critically evaluated the Ethiopian pharmaceutical regulatory framework from a broader international perspective, leading to some recommendations for policy strategies in order to restrict the problems related to poor quality medicines: strengthening the capacity of drug regulatory authorities for inspection activities; harmonization and regional sharing of information about manufacturing and distribution quality; enforcement of regulations and legal prosecutions; empowerment and capacity building of medicines inspectors; continuous monitoring of the different levels of medicines supply chain; continuous and sustainable product quality surveillance studies with strong corrective measures where required.

Experience gained from this PhD work made the Jimma University realize that capacity building for research is a prerequisite for developing a lasting research tradition and for ensuring that the research outcomes are translated into practical recommendations for decision makers to ultimately
improve the health of the citizens. Moreover, by establishing this medicine GMP QC-laboratory, the importance of quality systems has been generally recognized, and will thus not be limited to this medicine laboratory but will also overflow to other R&D university laboratories.

To summarize, this PhD thesis contributes to a better understanding of quality of medicines both from analytical and regulatory perspectives for possible evidence-based interventions in the process of medicines quality systems in Ethiopia and in the whole HOA region.

The outlook for the future resulting from this PhD work can be exemplified by following prospects:

1. Routine analytical QC services under GMP can be delivered to different stakeholders. Appropriate, rapid, stability-indicating and economical chromatographic analytical techniques were developed so that resource-limited economies in general and Ethiopia in particular could use them in the routine regulatory QC of medicines which are circulating in the markets. In addition, we are working with EFMHACA and other related national and international stakeholders to make JuLaDQ a center of excellence for drug quality both at national and regional level in the horn of Africa. Moreover, we are working recklessly with WHO for JuLaDQ WHO-prequalification as well as with the Ethiopian Ministry of Science and Technology for ISO-accreditation based on ISO/IEC 17025 quality management requirements. The first inspection results of both were encouraging. Finally, this GMP-aspect will also continuously be monitored and will be included in future research projects.

2. Further research and development activities on advanced medicines analytics. The advanced knowledge obtained from this PhD work and the establishment of JuLaDQ laboratory created a good opportunity for further R&D activities. Our research group would like to continue working in the following areas already started up:

   Quality evaluation of different essential medicines in Ethiopia and the HOA region: This study is a continuation of this PhD thesis and thus quality surveys of different medicines from essential drug list in Ethiopia will be conducted. Currently, quality survey of essential medicines including the anti-malarial drugs (quinine and chloroquine), antimicrobials (amoxicillin, norfloxacin, ciprofloxacin, and co-trimoxazole) and anthelminthic medicines is being conducted.

   The development of dissolution systems, including in-vitro/in-vivo correlation studies, on anti-malarial and anthelmintic medicines, seen their health importance and bioavailability issues. This study is a PhD project that has recently been started up, in a joint collaboration effort between Ghent and Jimma University.

   Analytical and bio-functional evaluation of different endemic plants of Ethiopia. Firstly, ethnobotanical studies will be conducted, endemic plants of Ethiopia with traditional claims of anti-infective property will be phytochemically screened and the molecular
characterization the different secondary metabolites responsible for the bio-functionality will be conducted. One PhD project on anti-malarial bio-functionality evaluation of the endemic plants of Ethiopia is already launched and preliminary results are being generated. We will extend this project to lead compound investigation and characterization towards optimized anti-infective agent discovery.

(3) Human resource capacity building.

We need to further develop human capacity with regard to quality analytics of medicines. The strategies are: (a) short-term training: continuous professional development in various areas of quality analytics of medicines, GMP/GLP and regulatory aspects; (b) long-term training: strengthening existing Master of Science (MSc) programs and launching new MSc and PhD programs in pharmaceutical quality sciences including student exchange programs.

(4) Scientific communications.

We will be involved in participating and organizing both national and international workshops and symposia on a wide range of drug quality and related fields of Pharmaceutical Sciences. Policy briefs will be communicated to the responsible regulatory authorities to improve the system and decisions. Research outputs will be communicated in reputable national and international journals.
“In all human affairs there are efforts, and there are results, and the strength of the effort is the measure of the result.”

James Allen
(*1864 - †1912, English author and poet)
SULTAN SULEMAN

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Surname: Suleman
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EDUCATION

1996 – 2001: Bachelor of Pharmacy (B.Pharm), School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia
2003 – 2005: Master of Science (M.Sc) in Pharmaceutical Analysis and Quality Assurance, School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia
2010: ‘Scientific Paper Writing’ at Jimma University, Jimma, Ethiopia
2010: ‘Transcriptomics’ of VIB Research and Training course at Ghent University, B-9000 Ghent, Belgium
2014: ‘Investigators Safety’ of Johnson-Johnson Pharma at Jimma University, Jimma, Ethiopia
2014: ‘Good Clinical Practice (GCP) and best Practices when Managing Clinical Trials’ of Johnson-Johnson Pharma at Jimma University, Jimma, Ethiopia
2014: ‘Interregional Seminar for Quality Control Laboratories involved in WHO Prequalification’, Potchefstroom, South Africa
2014: ‘Industrial Pharmacy Training’ of Gulf Pharmaceutical Industries, Ras Al Khaimah, United Arab Emirates

PROFESSIONAL EXPERIENCE

September 2001 – August 2002: Graduate Assistant II, School of Pharmacy, Jimma University, Jimma, Ethiopia
September 2002 – August 2003: Assistant Lecturer, School of Pharmacy, Jimma University, Jimma, Ethiopia
September 2005 – January 2009: Lecturer, School of Pharmacy, Jimma University, Jimma, Ethiopia
February 2009 – May 2014: Assistant Professor, School of Pharmacy, Jimma University, Jimma, Ethiopia
June 2014 – Present: Associate Professor, School of Pharmacy, Jimma University, Jimma, Ethiopia

POSITIONS

October 2005 – January 2008: Head, School of Pharmacy, Jimma University, Jimma, Ethiopia
April 2008 – March 2009: Deputy Leader (south), Infectious Disease and Modeling Project, VLIR IUC-JU Program, Jimma University, Jimma, Ethiopia
April 2010 – Present: Leader (south), Drug Quality and Therapeutics sub-project of Infectious Disease Epidemiology Project, VLIR IUC-JU Program, Jimma University, Jimma, Ethiopia

August 2015 – Present: Director, Jimma University Laboratory of Drug Quality (JuLaDQ), Jimma University, Jimma, Ethiopia

PUBLICATIONS IN JOURNALS WITH PEER REVIEW

PUBLISHED AND/OR ACCEPTED


**SUBMITTED**


**IN PREPARATION**

Suleman S, Belew S, Wynendaele E, Duchateau L, De Spiegeleer B. Quality-by-Design principles applied to the establishment of a pharmaceutical quality control laboratory in a resource-limited setting: lab-water system.

PRESENTATIONS AT (INTER)NATIONAL CONFERENCES

*Oral presentation*

*Poster presentation*

Suleman S, Baert B, Vangheluwe E, Duchateau L, De Spiegeleer B. Related Impurities profiling of anti-malarial drug lumefantrine. Scientific Afternoon in the Faculty of Pharmaceutical Sciences (FFW), May 2010, Faculty of Pharmaceutical Sciences, University of Ghent, 9000 Ghent, Belgium.  
*Poster presentation*

*Oral presentation*

*Poster presentation*

*Oral presentation*
Poster presentation

Suleman S, Vandercruiysen K, D'hondt M, De Spiegeleer B. A rapid stability-indicating fused core HPLC method for simultaneous determination of β-artermether and lumefantrine in anti-malarial fixed dose combination products. Scientific Afternoon in the Faculty of Pharmaceutical Sciences (FFW), May 2012, Faculty of Pharmacutical Sciences, University of Ghent, 9000 Ghent, Belgium.
Poster presentation

Oral presentation

Oral presentation

Poster presentation

Poster presentation

Poster presentation

*Poster presentation*

**PROFESSIONAL AND TRAVEL AWARDS**

**7 – 9 September 2013:** Gold Medal and Certificate of Merit of the year 2012 for the contribution to Pharmaceutical Research and Development in Ethiopia from Ethiopian Pharmaceutical Association (EPA), Addis Ababa, Ethiopia.

**31 August – 04 September 2008:** International Pharmaceutical Federation (FIP) full financial support to attend the FIP World Congress of Pharmacy and Pharmaceutical Sciences in Basel, Switzerland.

**3 – 8 September 2009:** International Pharmaceutical Federation (FIP) Congress Travel Grant award: The travel grant was awarded to attend the international pharmaceutical federation (FIP) congress in Istanbul, Turkey.