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JIMMA, ETHIOPIA

COMPARATIVE QUALITY ASSESSMENT OF DIFFERENT BRANDS OF METRONIDAZOLE AVAILABE IN JIMMA TOWN, SOUTH WEST ETHIOPIA

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DECLARATION

This is to certify that the research performed by Teshome Sosengo, entitled "Comparative quality assessment of different brands of Metronidazole available in Jimma town, South west Ethiopia" for partial fulfillment of requirements of Msc degree in pharmaceuticals quality assurance and regulatory affairs.

Here with my signature, I declare that this paper is done under my advisor ship and I have approved that this draft is the final paper for submission to the department of Pharmaceutical Quality Assurance and Regulatory Affairs, School of pharmacy, Jimma University.

Advisor:

Name……..signature……date……

Co-advisor:

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Here with my signature, I declare that this paper has been examined by me and I have checked that the student has incorporated the comments that I forwarded during examination.

Examiner:

Name……..signature……date……

Here with my signature, I declare that this research paper is done by me as a principal researcher and I assure that this paper is the final draft for submission to department of pharmacy , Jimma university.

Name……..signature……date……

ABSTRACT

Background: Poor quality drugs are worldwide problem with high prevalence in low and middle income countries. Metronidazole is BCS class I drug that was discovered in the 1960s. It is one of the commonly utilized antibiotics in Ethiopia. In spite of its broad use, poor quality Metronidazole has been frequently reported.

Objective: To assess the quality of different brands of Metronidazole available in Jimma town, Oromia regional state, South West Ethiopia.

Methods and materials: A cross sectional study was conducted to determine quality of different brands Metronidazole available in Jimma town from April 23-May 22, 2018. The quality of fourteen brands (three Metronidazole capsules, seven Metronidazole benzoate oral suspensions and four Metronidazole injections brands) of Metronidazole was assessed based up on a method specified in BP 2013 and USP 2015. The assay result of all the fourteen brands of Metronidazole and dissolution test result of the three brands of Metronidazole capsules was analyzed using statistical package for social sciences software version 24.0 to determine whether there exists significant difference in assay and dissolution test results of Metronidazole within and among the brands of the respective dosage forms using one way analysis of variance.

Result: All the fourteen brands of Metronidazole analyzed for quality passed identity test specification of BP 2013. The three brands of Metronidazole capsules passed weight uniformity and dissolution test specification of USP 2015. The highest weight variation and dissolution, 9.218% and 106.61%, obtained for Metronidazole (generic) and Camezol respectively. All the fourteen brands of Metronidazole analyzed for quality passed assay (i.e. drug content) test specification of USP 2015. The highest percentage of drug content, 107.81%, 101.03% and 105.56%, obtained for Metronidazole (generic)(capsule), Metrolag (suspension) and Nirmet(injection) respectively. However, statistical comparison of assay of respective brands of the respective dosage forms at 95% confidence interval indicates that there exists significant difference in assay within and among the brands of the respective dosage forms ($p<0.05$). The pH of all brands of Metronidazole benzoate oral suspensions and Metronidazole injections were within BP 2013 specification range.

The seven brands of Metronidazole benzoate oral suspensions passed total aerobic microbial test with highest number of colony forming units, each 20 CFU/ml, obtained for Camezol and

Metrogyl. All the four brands of Metronidazole injections passed USP 2015 limit for Endotoxin, Endotoxin limit <0.35Eu/ml. Two brands of Metronidazole injection, Aldezol and Metris, failed sterility test from the four brands of Metronidazole injections included in the study and hence of poor quality.

Conclusion and recommendation: The result of the current study revealed that there was incidence of poor quality Metronidazole in Jimma town. Therefore, post marketing quality assessment should be performed routinely to determine quality status of the drug on market.

Key words: poor quality drugs, substandard drugs, falsified drugs, counterfeit drugs, quality.

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ABBREVIATIONS AND ACRONYMS

API: Active pharmaceutical ingredient

EFMHACA: Food, Medicines and Health care administration and control authority

BET: Bacterial Endotoxin test

BP: British Pharmacopoeia

CFU: Colony forming units

EPHARM: Ethiopian pharmaceutical manufacturing share company

FTIR: Fourier transform infrared spectroscopy

GMP: Good manufacturing practice

HPLC: High performance liquid chromatography

ICRS: International chemical reference substance

IPA: Isopropyl alcohol

LAL: Limulus amoebocyte lysate

NMRAs: National medicines regulatory authorities

NMT: Not more than

PDA: Photo diode array detector

pH: Power of hydrogen

RS: Reference standard

RSD: Relative standard deviation

TSA: Tryptone soya agar

USD: United States dollar

USP: United States Pharmacopeia

WHO: World health organization

1. INTRODUCTION

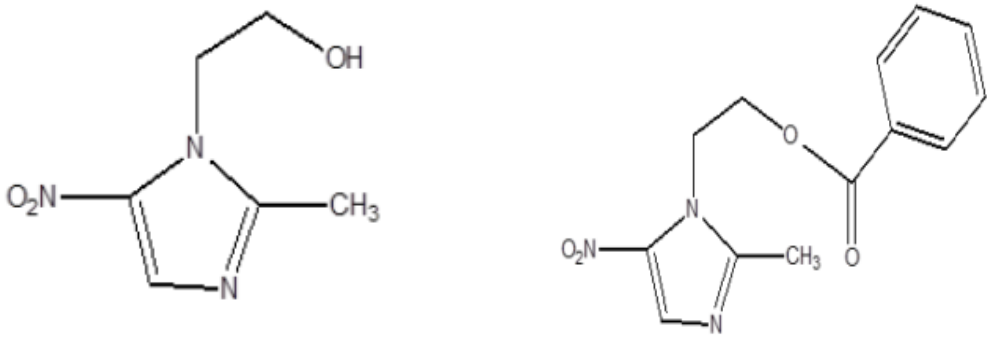
1.1. Background

Poor quality drugs are worldwide problem with high prevalence in low and middle income countries (WHO, 2017 ; Almuzain et al., 2013). WHO report of 2017 estimates that the rates of substandard and falsified medical products in low- and middle-income countries is approximately 10.5% with an estimated spend of US$ 30.5 billion (WHO, 2017). Poor quality medicines include substandard and falsified medicines. Substandard medical products are authorized medical products that fail to meet either their quality standards or their specifications or both. Falsified medical products are deliberately/ fraudulently miss-present with respect to identity, composition or source (WHO, 2017).

Poor quality pharmaceuticals invade health care system because of a number of problems starting from manufacture to final use by patients. Non adherence to good practices in manufacturing, storage, distribution and dispensing, weak enforcement of pharmaceuticals regulatory laws, open borders, poor coordination of police and customs, corruption, double standards during production of pharmaceuticals i.e. better standards for manufacture of drugs to be exported rich countries and the poor standard for to be exported to poor countries as Sub-Saharan African countries and low educational level results in invasion of the health care system with poor quality pharmaceuticals (Bate et al, 2014 ; Caudron et al., 2013 ; WHO, 1999 ; SPS, 2011 ; Kaur et al, 2015).

Metronidazole is [1-(2 hydroxyethyl)-2-methyl-5-nitroimidazole] that was discovered in the 1960s (Rediguieri et al., 2011). Since from its discovery, it is used in treatment of numerous infections caused by bacteroides, clostridia, helicobacter, trichomonas, giardia and entamoeba, dental infections, skin infections, surgical prophylaxis and antibiotic associated pseudomembraneous colitis (Samuelson, 1999 ; Hedge et al., 2008 ; Kissinger, 2015 ; Gardner and Hill, 2001 ; Bansal et al., 2006 ; Raza et al., 2005 ; Peedikayil, 2016 ; Dhand and Snydman, 2014). The chemical structure is shown below in figure 1. Metronidazole is BCS class I drug, drugs whose more than 85% of the drug content should dissolve within 30 min so that dissolution shall not become the rate-limiting step for the absorption of the drug (Rediguieri et al, 2011). It is one of the commonly utilized antibiotic in Ethiopia (Abrha et al., 2015 ; Erku et al., 2017).

In spite of its broad use, poor quality Metronidazole has been frequently reported (Tschida, 2016 ; Taylor et al., 2001 ; Ibezim et al., 2008 ; Adil et al., 2016 ; Löbenberg et al., 2012).



A) Metronidazole B) Metronidazole benzoate

Figure 1: Chemical structure of Metronidazole and Metronidazole benzoate.

1.2. Statement of problem

Poor quality medicine is a global health problem (WHO, 2017 ; Bate, 2011). WHO global surveillance and monitoring system for substandard and falsified medicines has received more than 1500 reports of substandard or falsified medicines, with 42% of reports coming from sub-Saharan Africa, and 21% each coming from the Americas and Europe in the years from 2013-2017. During the years, 8% of reports came from the Western Pacific, 6% from the Eastern Mediterranean and 2% from South-East Asia (WHO, 2017).

The problem of poor quality medicines affects almost all categories of drugs (Kelesidis et al., 2007 ; WHO, 2017). Antibiotics and anti-malarias are most commonly reported poor quality drugs(WHO, 2017 ; Kelesidis and Falagas, 2015). Almuzani et al on his systematic literature review of substandard and counterfeit medicines, performed on 15 studies conducted in 25 different countries, mainly in Africa and Asia, report out that 28.5% of the samples included in the studies were of poor quality. Antibiotics being the most commonly reported poor quality drugs followed by anti-malarial drugs (Almuzani et al., 2013).

Poor quality pharmaceuticals frequently fail to meet critical quality attributes specification limit set for them when tested for quality. Failure to comply with API, dissolution and disintegration specification is most commonly reported problems in poor quality pharmaceuticals (Algahannam et al., 2014 ; Almuzain et al., 2013 ; Sammons, 2017 ; Bate R et al., 2014).

Treatment with poor quality pharmaceuticals causes deleterious problems as treatment failure, increased morbidity and mortality, wastage of budget of family and government and emergence of drug resistance (Newton et al., 2009 ; Alhedethe et al., 2014 ; Kelesidis & Falagas, 2015 ; Wilson, 2017 ; WHO, 2017). A standard dose kills drug susceptible strains of microbes and suppresses multiplication of the drug resistant microbes. Substandard medicines selectively kill the susceptible strain and leaves resistant strain to multiply (WHO, 2017 ; Newton et al, 2016). Micro-organisms that have developed resistance transmits resistance gene through exchange of genetic material (Pisan, 2015). An estimated 700,000 Africans die annually from consuming fake anti-malarial or tuberculosis drugs (Wilson, 2011). In Sub-Saharan Africa an estimated 400,000 children are exposed to malaria are treated with poor quality anti-malaria medicines (Seither, 2009). In Panama, cough syrup with deliberately mislabeled ingredient—Diethylene glycol instead of glycerin killed about 200 people (Seither, 2009).

Systematic literature review done in Japan on 86 studies found out that Metronidazole was the second most repeatedly reported substandard antibiotic next to Cloxacillin and from the 277 samples of Metronidazole included in the literature review, 69(24.9%) of the Metronidazole samples were substandard(Tschida, 2016). Treatment with substandard dose of Metronidazole causes emergence of drug resistance and treatment failure (Rasoloson et al., 2002 ; van der Wouden, 1997).

In Ethiopia, there exists poor co-ordination of police and custom, a factor that results in invasion of the health care system by poor quality pharmaceuticals such as counterfeit pharmaceuticals and hence in exposure of the patients, community and the government to the deleterious impacts of such poor quality pharmaceuticals (Suleman et al., 2016). Study conducted in Ethiopia by Suleman et al on 106 samples of Albendazole, Mebendazole and Tinidazole tablets, medicines commonly used in the treatment of soil transmitted helminthes and giardia, indicate that (48.0%, 95% CI: 28.4 to 67.6) of Albendazole tablets, (45.2%, 95% CI: 30.2 to 60.3) of Mebendazole tablets and (43.6%, 95% CI: 28.0 to 59.2) Tinidazole tablets were of poor quality. Overall, 45% (48/106) of the drug samples analyzed were found to be of poor quality (Suleman et al., 2014).

To estimate the exact burden of poor quality medicines and formulate effective and efficient strategy to prevent exposure of the patient, community, government and the health care system from poor quality drugs and hence from its effects, there should plenty of data on poor quality drugs. To date, few data are available on poor quality drugs (Newton et al., 2009 ; WHO, 2017 ; Almuzani et al., 2013). In Ethiopia, except a few attempts of certain scholars to assess quality of certain drugs circulating on the pharmaceutical market of the country, the quality status of majority of the drugs circulating in the health care system of the country yet remain unknown. Therefore, this study assesses the quality of different brands of Metronidazole marketed in Jimma town.

1.3. Significance of the study

Poor quality drugs are common in countries with weak regulatory systems and hence the patients, government, the health care system and community are exposed to the deadly effects of the drugs (Bate et al., 2014 ; Pisan, 2015; Christian et al., 2012). Pharmaceuticals regulatory system of Sub-Saharan African countries is weak (SPS, 2011). In Ethiopia, there exists weak enforcement of regulations to control entry and distribution of pharmaceuticals, a factor that results in invasion of the pharmaceutical market by poor quality pharmaceuticals (Suleman S et al., 2016 ; Christian et al., 2012).

To date, in Ethiopia, except a few attempt to assess the quality of Metronidazole in Addis Ababa, the capital (Kahaliw and Ashenafi, 2013), the quality status of Metronidazole marketed in areas far from the capital as Jimma town is yet remain unknown. Therefore, this study assesses quality of different brands of Metronidazole capsules, injections and Metronidazole benzoate oral suspensions marketed in Jimma town. The findings from this study shall serve as a baseline for studies that shall be done to assess quality of drugs, input for policy formulators to formulate appropriate policy to prevent prevalence of poor quality medicines and will also contribute to the body of knowledge on post-market quality of drugs.

2. LITERATURE REVIEW

Poor quality Metronidazole has been reported in studies done in different countries of the world. In 1999, WHO conducted a cross-sectional study in Myanmar and Viet Nam and found out that from 212 samples tested from Myanmar (amoxicillin(25), Ampicillin(22), Chloramphenicol(23), Chloroquine(9), Cotrimoxazole(21), Metronidazole(14), Paracetamol(44), ranitidine(25), Rifampicin(11), Tetracycline(18)), 7.1 % of the samples of Metronidazole contain only 25-60% of labeled amount active pharmaceutical ingredient and hence failed BP 93 and USP 23 specification for content of active pharmaceutical ingredient based on which the samples was assessed for quality and 10 of the Metronidazole samples were not registered in the country (WHO,1999).

A cross-sectional study based on stratified random sampling done in China on 506 samples of antibiotics, (Amoxicillin(114), Levofloxacillin(101), Cefuroximeaxetil(91), Metronidazole(108) and Azithromycin(92)), revealed out that from 108 samples of Metronidazole analyzed on the study, 41(38%) of the Metronidazole samples failed assay test specification of Chinese pharmacopoeia version 2010, 93-107% of API, based up on which they are evaluated for quality (Pan H et al., 2016).

Ahmed F et al conducted a quality assessment study in Bangladesh in 2003 on 30 brands of Metronidazole tablets and ten brands of Metronidazole suspensions to assess quality of marketed Metronidazole preparations and report out that four brands of Metronidazole tablets and two brands of Metronidazole suspensions fail to meet BP specification for potency (Ahmed, 2003).

In Pakistan, each two brands of Metronidazole tablets failed disintegration, dissolution and friability tests respectively in a comparative quality assessment study performed by Adil M et al in Peshwar, Pakistan, on thirteen brands of Metronidazole tablets. Only four brands passed all the tests as per USP pharmacopoeia specification, a standard based on which the brands were tested for quality, while the rest of the brands did not fulfill the standards specification and failed at least one test (Adil et al., 2016).

In a microbiological quality assessment study done in Sri Lanka on five drugs (Paracetamol, Salbutamol, Cephalexin, amoxicillin and Lactulose), each having three brands, to assess microbiological quality of pediatric oral liquid drug formulations during consumption, all the three brands of lactulose was found to be contaminated with microbes (Sudeshika et al, 2014).

In Karachi, Pakistan, a study done by Fatima S et al on three brands of Metronidazole tablet formulations, two test brands and one innovator brands, to test therapeutic equivalence of Metronidazole immediate release tablets, indicated that except the innovator brand the two test brands of the study failed dissolution test (Fatima et al., 2017).

A cross-section study based on random sampling was conducted by Khurelbat et al on 1236 samples (388 samples from rural area and 848 samples from urban district, in which 1 sample corresponds 100 dosage unit (i.e. tablet or capsule)) of (Metronidazole tab, Nystatin tab, Ibuprofen, Cotrimoxazole tab, Amoxicillin cap, Paracetamol tab, Ampicillin cap, Bromhexin tab and Doxycycline cap) in Mongolia, find out that 16%(47) Metronidazole tablets from 287 tablets Metronidazole tablets included in the study from rural area and 2 %(17) from 763 Metronidazole tablets analyzed from the urban district were of unacceptable quality. In the study, (6 %(17), 1%(2), 5%(15), and 4%(12)) of Metronidazole failed assay for active pharmaceutical ingredient, dissolution, and weight variation respectively from rural area and from urban area, each 1%(5) of the drug failed test for assay for active pharmaceutical ingredient and dissolution (Khurelbat et al., 2014).

In a study carried out in Eastern Nigeria to evaluate microbial and physicochemical qualities of Co-trimoxazole and Metronidazole formulations, one brand from eight brands of Metronidazole suspensions and three brands from nine brands of Co-trimoxazole suspensions failed microbiological quality assessment test (Nwakile et al., 2011).

Taylor et al on his analysis of 581 drug samples of 27 different drugs, on which the drug samples was collected from Lagos and Abuja, Nigeria, showed that from 36 Metronidazole tablets and 5 Metronidazole suspension included in the study, 26(72%) of Metronidazole tablets failed BP specification and all the 5 Metronidazole suspensions did not contain API (Taylor et al., 2001).

In Nigeria, in a study carried out by Ibezim et al for in vitro prediction of in vivo bioavailability and bioequivalence of different brands of Metronidazole available in Eastern Nigerian drug market, only two batches of Metronidazole demonstrated evidence of bioequivalence from ten batches of Metronidazole tablets analyzed. In addition, only three brands of Metronidazole passed potency specification and three brands failed dissolution test specification of BP, standard based up on which the brands were evaluated for quality. All the brands passed a test for weight uniformity in the study (Ibezim et al., 2008).

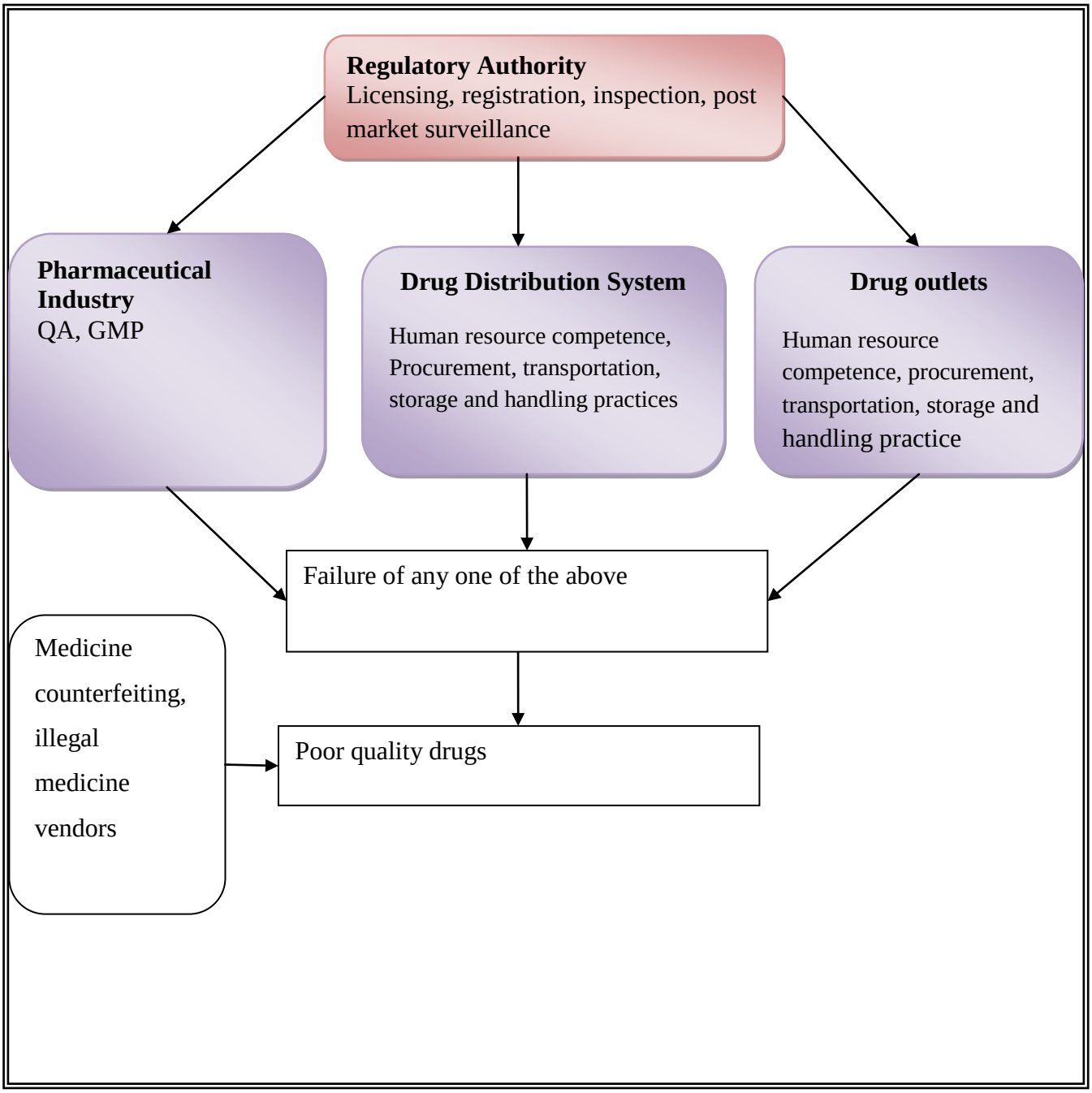
A study conducted on randomly selected 15 brands of Metronidazole tablets to assess physico-chemical properties of Metronidazole tablets marketed in Zaria, Nigeria, indicated that only 60% of the sampled drugs passed the quality control tests. All the brands complied with the weight uniformity, disintegration, potency, dissolution and chemical identification test specification of BP, standard based upon which the brands was tested for quality (Musa et al., 2011).

In Lagos, Nigeria, a study undertaken on thirteen brands of Metronidazole tablets, where one of the brand is an innovator generic brand, for comparative assessment of generic Metronidazole tablets commercially available in Lagos, Nigeria, revealed out that four brands of Metronidazole tablets were not equivalent to the innovator brand and hence cannot be interchangeably used with the innovator brand (Ilomuanya et al., 2015).

A cross-sectional study based on random sampling performed in Dar Es Salaam, Tanzania on twenty-four samples of quinine syrup and three batches of water for injection to evaluate microbiological quality of commercially available quinine syrups and water for injection in Dar Es Salaam, Tanzania, showed that all the three batches of water for injections included in the study was found to be contaminated with pyrogens. In the study, all the quinine syrup samples included in study complied with microbial limit specification of USP (Mwambete et al., 2009).

A cross-sectional study performed by Mugoyela V and Mwambete K D in Dar Es Salaam, Tanzania to evaluate microbial contamination of 10 non-sterile pharmaceuticals found out that 50% of all tested products were contaminated with lethal microbes (Mugoyela and Mwambete, 2010).

2.1. Conceptual framework



3. OBJECTIVES

3.1. General objective

To assess the quality of Metronidazole dosage forms available in Jimma town, Oromia regional state, South West Ethiopia.

3.2. Specific objectives

- To identify Metronidazole and Metronidazole benzoate in different brands of Metronidazole capsules, injections and Metronidazole benzoate oral suspensions.
- To determine the drug content (assay) of different brands of Metronidazole capsules, injections and Metronidazole benzoate oral suspensions.
- To assess the weight uniformity of different brands of Metronidazole capsules.
- To evaluate the *in-vitro* dissolution profiles of selected brands of Metronidazole capsules.
- To assess the microbiological quality of different brands Metronidazole benzoate oral suspensions and Metronidazole injections.

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4. METHODS AND MATERIALS

4.1. Study area and period

The study was conducted in Jimma town, Oromia regional state, Ethiopia. It is located 352 km South West of Addis Ababa. Currently in Jimma town there exits 33 drug stores and 22 pharmacies serving the population. The study was conducted from April 23 – May 22, 2018.

4.2. Study design

A cross sectional study was conducted to determine quality of Metronidazole available in Jimma town.

4.3. Population

4.3.1. Source population

All brands of all dosage forms of Metronidazole available in retail outlets (i.e. private pharmacies and drug stores) found in Jimma town were source population for the study population.

4.3.2. Study population

All brands of all dosage forms of Metronidazole available in retail outlets (i.e. private pharmacies and drug stores) found in Jimma town during study period i.e. from April 23-May 22, 2018 that fulfill the inclusion criteria.

4.4. Sample size determination

Eighty capsules from each brand of Metronidazole capsules, each four bottles from each brand of Metronidazole injections and Metronidazole benzoate oral suspensions were purchased.

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

- All dosage forms of Metronidazole that have more than one brands.

4.5.2. Exclusion criteria

- Dosage forms of Metronidazole which has only one brand.
- Brands that was not available on the market during sample collection period.

Hence, fourteen brands of Metronidazole (three Metronidazole capsules, seven Metronidazole benzoate oral suspensions and four Metronidazole injections brands) were included in the study.

4.6. Sample collection technique

Samples were collected using convenience sampling technique (WHO, 2015 ; Etikan et al, 2016). Detailed information of the samples purchased for analysis of quality on the present study is indicated in tables 1, 2 and 3.

Table 1: Detailed information of Metronidazole 250 mg capsules analyzed for quality.

Manufacturer	Brand name	Strength	Batch N^{o}	Mfg.date	Exp.date
EPHARM. Ltd., Ethiopia	Metronidazole (generic)	250mg	BN712018	07/2017	12/2021
Cadila Pharmaceuticals Plc, India	Camezol	250mg	D17062bx56	12/2017	11/2020
Addis Pharmaceuticals Factory Plc, Ethiopia	Metazol	250mg	BN23336	-	06/21

Table 2: Detailed information of Metronidazole benzoate oral suspensions analyzed for quality.

Manufacturer	Brand Name	Strength	Batch Nº	Mfg.date	Exp.date
Neopharma, UAE	Metrolag	125mg/5ml	MZA16007	05/2016	05/2019
Unique Pharmaceuticals Labs., India	Metrogyl	125mg/5ml	ASX7001	Nov.2017	Oct.2019
Coral Laboratories Ltd., India	Cornizole	200mg/5ml	DCI1715	08/2017	07/2020
Julphar Pharmaceuticals Plc., Ethiopia	Negazole	125mg/5ml	0020	0/2017	09/2020
Fawes Pharmaceuticlas Plc., Ethiopia	Mizel	125mg/5ml	18005912	04/2018	04/2020
Cadila Pharmaceuticals Plc,India	Camezol	125mg/5ml	D17004BY37	Jul.2017	Jun.2020
Addis Pharmaceuticals Factory Plc.,Ethiopia	Metazol	125mg/5ml	24168	10/2017	10/2020

Table 3: Detailed information of Metronidazole injections analyzed for quality.

Manufacturer	Brand Name	Strength	Batch Nº	Mfg.date	Exp.date
Aculife Health care Pvt.Ltd., India	Nirmet	500mg/100ml	2H61981	Aug.2016	Jul.2019
Unique Pharmaceuticals Labs., India	Metrogyl	500mg/100ml	Plx7070	04/2017	03/2020
Albert David Ltd., India	Aldezol	500mg/100ml	P6130023E	06/2016	05/2019
Claris Otsuka Pvt., India	Metris	500mg/100ml	C262629	10/2016	09/2019

4.7. Equipments

FTIR 8400S (code: A21014100360LP,SHIMADZU, Japan), HPLC (Mode:LC-2030C3D,Japan), dissolution tester (code: DT13460835, India), UV-Spectrophotometer (code: A11455401989CD, Shmadzu, Japan), sonicator (Bandelin, Germany), thermometer(Frankfurt, Germany), volumetric flask (England), pycnometer (Germany), Whatman GFC paper (England), conical flask (MERK, Germany), evaporating dish (Britain), 0.45µm Nylon membrane filter (Germany), analytical balance (METLER TOLEDO, Switzerland), pH meter (Metler Toledo, China), incubator (SANYO, Japan and FOC225E, Turkey), heating oven (Eclipse, Italy), sterile syringe (China) and KBr plate (Britain).

4.8. Solvents/chemicals/reagents

Methanol (CARLOERBA, France), glacial acetic acid (CARLOERBA, France), 1M phosphoric acid (Germany), 0.1 N HCl, Nacl (Fischer scientific, UK), monobasic potassium phosphate (CARLOERBA, France), thioglycolate medium (Himedia laboratory Pvt.Ltd, India), Isopropyl alcohol (National Alcohol, Ethiopia), acetone (CARLOERBA, France), soybean casein digest medium (Himedia laboratory Pvt.Ltd, India), LAL reagent (Charles River, India), tryptone soya agar (Sisco reasercher Laboratory, India), water for bacterial Endotoxin test (Nearlite, India), Endotoxin reagent (Charles reagent, India), distilled water (EPHARM, Ethiopia). Metronidazole ICRS (Lot number: 183118, WHO center for chemical reference substances, Sweden) was obtained from EPHARM and Metronidazole benzoate BP RS (lot no. MBO/15120558 with potency of 99.9 %) was obtained from EFMHACA.

4.9. Quality Evaluation

4.9.1. Metronidazole capsule

4.9.1.1. Identification test

4.9.1.1.1. Sample preparation

First, the content of 10 capsules was mixed. Then, 10 mg of Metronidazole sample was taken in KBr plate to the FTIR instrument for the identification test (BP, 2013).

4.9.1.1.2. Analysis

First, 10 mg of Metronidazole ICRS was placed on KBr plate and taken to the FTIR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1}. Consequently, 10mg of Metronidazole sample was taken with KBr plate to IR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1} (BP, 2013).

Acceptance criteria: The IR absorption spectrum of the standard and the sample under study should coincide

4.9.1.2. Weight uniformity test

First, 10 capsules were selected randomly (USP, 2015). Then, each intact capsule was weighed and its shell was opened and its content removed. The empty shell was weighed and the net weight of the content of the each capsule was determined by subtracting the weight of the shells from the weight of the intact capsule. The procedure was repeated for the remaining 9 capsules. Then, the average net weight was determined for the 10 capsules. Then, assay of individual capsule (% xi), was obtained by formula:

$$\%\text{xi} = \frac{\text{A} \times \text{Wi}}{\text{av.wt}} \times 100\% \text{ (USP, 2015).}$$

Where, A= total percent assay which is obtained from assay done on representative drug samples of each brand as indicated on monograph of the brands.

wi= net weight of each capsule

av.wt= average net weight of ten capsules.

Finally, average % xi for ten capsules was determined by dividing the sum of individual capsule assay by 10, which is the number of capsules used for weight uniformity test in the study (USP, 2015).

Acceptance value (AV): Acceptance value was determined by formula:

$$\text{AV} = \left| \text{av.}\,\%\,\text{xi} - \text{M} \right| + \text{ks}.$$

Where,

M =reference value which 101.5% for average %xi assay for the brands is greater than 101.5%.

k= acceptability constant which is 2.4, for number of capsules is 10.

s= standard deviation of % xi, calculated by the formula: $s = [\sum_{n=1}^{n}(xi - av.\ \%\ xi)^2]^{1/2}$.

Acceptance criteria: L1 (maximum allowed acceptance) = 15.0% (USP, 2015).

4.9.1.3. Assay test

4.9.1.3.1. Preparation of mobile phase

Mobile phase of 1000 ml was prepared by mixing 250 ml of methanol in 750 ml of water in 1000ml volumetric flask (USP, 2015).

4.9.1.3.2. Preparation of standard solution

Standard solution of strength 0.03mg/ml was prepared by dissolving 3 mg of Metronidazole ICRS in 100 ml of mobile phase (USP, 2015).

4.9.1.3.3. Preparation of sample stock solution

First, the content of 20 capsules was mixed. Then, an amount equivalent to 100 mg of Metronidazole was transferred to a 100 ml of volumetric flask and 80 ml of mobile phase was added to it and the mixture was sonicated with intermittent shacking for 10 minutes. Then, the mixture was shacked for 30 minutes and diluted with mobile phase to a volume resulting in formation of sample stock solution of strength 1mg/ml. Then, 30 ml of the solution was centrifuged (USP, 2015).

4.9.1.3.4. Preparation of sample solution

To prepare sample solution, first 1.5ml of sample stock solution was transferred to 50 ml volumetric flask and diluted with the mobile phase to a volume resulting in formation of sample solution of strength 0.03mg/ml. Then, 30 ml of the solution was filtered through 0.45 µm nylon membrane filter and the first 10 ml of the filtrate was discarded and the remaining 20 ml was used for the analysis (USP, 2015).

4.9.1.3.5. Chromatographic system

An Agilent HPLC system (a column: 4.6mm x 15cm; with 5µm L7 internal packaging, detector: PDA detector (λmax) =319 nm)) was used. The column temperature was fixed at 30°C and the flow rate was maintained at 1.0ml/min.

4.9.1.3.6. System Suitability

Six replicate injection of 30µl of standard preparations were injected to the HPLC.

Acceptance criteria: Relative standard deviation for six replicate injections should be NMT 2% and a tailing factor NMT 2 **(Table 4)**.

Table 4: System suitability test result of Metronidazole capsules (n=6).

<< PDA >>
ID#1 Compound Name: Metronidazole

Title	ample Nam	Sample ID	Ret. Time	Area	Height	Tailing Factor	er of Theoretical Plate	Conc.
C RS - 01.lc	Metronidaz	C RS - 01	4.286	2692195	319012	1.276	5507	100.000
C RS - 02.lc	Metronidaz	C RS - 02	4.271	2691171	320988	1.261	5546	99.981
C RS - 03.lc	Metronidaz	C RS - 03	4.256	2689967	324621	1.249	5551	99.957
C RS - 04.lc	Metronidaz	C RS - 04	4.238	2683069	326389	1.239	5594	99.776
C RS - 05.lc	Metronidaz	C RS - 05	4.225	2673500	327111	1.234	5543	99.535
C RS - 06.lc	Metronidaz	C RS - 06	4.213	2661592	327565	1.230	5501	99.242
Average			4.248	2681916	324281	1.248	5540	99.749
%RSD			0.654	0.454	1.086	1.392	0.608	0.305

Run time: 8 minutes.

4.9.1.3.7. Analysis

Three replicate 30µl sample solutions were injected automatically to the HPLC. Then, the percentage of the labeled amount of Metronidazole in the brands was calculated by the formula:

$$\text{Result} = (\text{ru}/\text{rs}) \times \left(\frac{\text{Cs}}{\text{Cu}}\right) \times 100\% \text{ (USP, 2015).}$$

Where,

$\text{ru} = \text{peak response from the sample solution}$

$\text{rs} = \text{peak response from the standard solution}$

$\text{Cs} = \text{concentration of USP Metronidazole RS in the standard solution(mg/ml)}$

$Cu = \text{nominal concentration of Metronidazole in the sample solution}(mg/ml)$

Acceptance criteria: 90.0% -110.0% (USP, 2015).

4.9.1.4. Dissolution test

4.9.1.4.1. Preparation of standard stock solution

First, 40 mg of Metronidazole ICRS was dissolved in 100ml of 0.1 N HCl. Then, the resulting mixture was sonicated resulting in formation of standard stock solution of strength 0.4mg/ml (USP, 2015).

4.9.1.4.2. Preparation of standard solution

To prepare standard solution, first 2ml of standard stock solution was transferred to 50 ml volumetric flask and diluted to a volume with 0.1N HCl. Then, the resulting solution was sonicated resulting in formation of standard solution of strength 0.016mg/ml.

4.9.1.4.3. Preparation of sample solution

Dissolution testing was performed using a USP Apparatus I (USP, 2015). The paddle was set at 100 rpm and 900 ml dissolution 0.1N HCl was used to test all samples. Prior to dissolution testing, the dissolution media was preheated and degassed. Dissolution testing was started after the temperature of 37ºC±0.5 ºC was confirmed in all six vessels. In pre-set time points of 10, 15, 20, 30, 45 and 60 minutes, 5ml of the sample solution was taken with syringe from the dissolution vessel and filtered through 0.45µm membrane filter. Then, the first 2ml was discarded and the remaining 3 ml of the filtrate was transferred to 50 ml volumetric flask and diluted to a volume with 0.1N HCl (USP, 2015).

4.9.1.4.4. Instrumental condition

First, UV spectrophotometer wavelength was filled to λmax of 278 nm (USP, 2015). Then, the reading of UV spectrophotometer was checked that it reads to zero in air to air reading. Then, two empty Quartz cuvets was selected and rinsed first with distilled water and then with 0.1 N HCl and placed in UV spectrophotometer and checked that the spectrophotometer reads zero. Then, the two Quartz cuvets was taken from the UV spectrophotometer and filled with the blank (i.e. 0.1N HCl) and placed in to UV spectrophotometer and the reading of UV spectrophotometer was once adjusted to zero. Then, while leaving one of the blank filled

Quartz cuvets in the spectrophotometer, the second was taken and used for placing the standard and sample solution in to the spectrophotometer for analysis.

4.9.1.4.5. Analysis

First, 1.5 ml of the standard solution was taken to the UV spectrophotometer and its reading was recorded and found to be 0.6061. Subsequently, 1.5 ml the sample solution was placed in Quartz cuvet and put in to UV spectrophotometer and its reading was recorded. Finally, the percentage of Metronidazole that was dissolved was calculated by formula:

$$\text{Result} = \left(\frac{\text{Au}}{\text{As}}\right) \times \text{Cs} \times \left(\frac{1}{\text{L}}\right) \times \text{V} \times 100\% \text{ (USP, 2015).}$$

Where,

$\text{Au} = \text{absorbance of the sample solution}$

$\text{As} = \text{absorbace of the standard solution}$

$\text{Cs} = \text{concentration of USP Metronidazole RS in the standard solution } \left(\frac{\text{mg}}{\text{ml}}\right)$

$\text{L} = \text{label claim } \left(\frac{\text{mg}}{\text{capsule}}\right)$

$\text{V} = \text{volume of medium, } 900\text{ml}$

Tolerances: NLT 85% of the labeled amount of Metronidazole dissolved in 30 minutes (USP, 2015).

4.9.2. Metronidazole benzoate oral suspension

4.9.2.1. Identification test

4.9.2.1.1. Preparation of sample solution

The volume of oral suspension containing of 200 mg of Metronidazole benzoate and 20ml of water was mixed in 250 ml conical flask (BP, 2013). The resulting mixture was mixed by shaking and filtered under partial vacuum. Then, the residue was washed with three 10 ml of water and dissolved in 10 ml of acetone in conical flask and filtered through Whatman GFC paper having pore size of 0.45µm. Then, the filtrate dried in an evaporating dish and 10 mg of the sample was taken in KBr plate to FTIR instrument for the identification test (BP, 2013).

4.9.2.1.2. Analysis

First, 10 mg of Metronidazole ICRS was placed on KBr plate and taken to the FTIR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1}. Consequently, 10mg of Metronidazole sample was taken with KBr plate to IR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1} (BP, 2013).

Acceptance criteria: The IR absorption spectrum of the standard and the samples under study should coincide.

4.9.2.2. Assay test

4.9.2.2.1. Preparation of solution A

1000 ml of Solution A of 0.1 % (v/v) of glacial acetic acid in water was prepared (USP, 2015).

4.9.2.2.2. Preparation of mobile phase

Mobile phase of 1000 ml volume was prepared by mixing of 400ml Acetonitrile and 600 ml of solution A. Then, the solution was filtered and degassed (USP, 2015).

4.9.2.2.3. Preparation of standard stock solution

Standard stock solution was prepared by dissolving 40 mg of Metronidazole benzoate BP RS in 100ml mobile phase. Then, the mixture was mixed by shaking resulting in formation of standard solution of strength 0.4mg/ml (USP, 2015).

4.9.2.2.4. Preparation of standard solution

To prepare standard solution, 25 ml of standard stock solution was transferred to 50 ml volumetric flask and the diluted to volume with the mobile phase and the resulting mixture was mixed by shacking resulting in formation of standard solution of strength 0.2mg/ml.

4.9.2.2.5. Preparation of sample solution

4.9.2.2.5.1. Preparation of sample solution of the six brands

To prepare sample solution of six brands Metronidazole benzoate oral suspensions, except Cornizole, first each bottle of the suspensions was shacked. Then, 0.8 ml, the volume

containing 20mg of the Metronidazole benzoate, was transferred to a 100 ml volumetric flask and diluted to a volume with a mobile phase. The resulting mixture was mixed by shaking resulting in formation of sample solution of strength 0.2mg/ml (USP, 2015).

4.9.2.2.5.2. Preparation of sample stock solution of Cornizole

To prepare sample stock solution of Cornizole, first the bottle of Cornizole was shaken. Then, 0.8 ml of Cornizole suspension, the volume that contains 32 mg of Metronidazole benzoate, was taken to 100 ml volumetric flask and diluted with the mobile phase to a volume. The resulting mixture was mixed by shaking resulting in formation of sample stock solution of strength 0.32mg/ml.

4.9.2.2.5.3. Preparation of sample solution of Cornizole

To prepare sample solution of Cornizole, 15.6 ml of Cornizole stock solution was taken to 25 ml volumetric flask and diluted to a volume with mobile phase. The resulting mixture was mixed by shaking resulting in formation of sample solution of strength 0. 2mg/ml.

4.9.2.2.6. Chromatographic system

An Agilent HPLC system (a column: 4.6mm x 15cm; with 5µm internal packaging L1, detector: PDA (λmax= 316 nm)) was used. The column temperature was fixed at 30°C and the flow rate was maintained at 1.0ml/min.

4.9.2.2.7. System Suitability

Six replicate 5µl of standard solutions were injected to the HPLC to ascertain whether requirements for precision are met.

Acceptance criteria: Relative standard deviation for six replicate injections should be NMT 2% and a tailing factor NMT 2 **(Table 5)**.

Table 5: System suitability test result of Metronidazole benzoate oral suspensions (n=6).

<< PDA >>
ID#1 Compound Name: Metronidazole

Title	Sample Nam	Sample ID	Ret. Time	Area	Height	Tailing Factor	er of Theoretical Plate	Conc.
W RS-01.lcd	Metronidazo	W RS-01	7.342	3005278	341705	1.233	14566	100.000
W RS-02.lcd	Metronidazo	W RS-02	7.344	3009359	340947	1.233	14589	100.068
W RS-03.lcd	Metronidazo	W RS-03	7.342	3003527	341879	1.233	14574	99.916
W RS-04.lcd	Metronidazo	W RS-04	7.342	3003879	342147	1.233	14656	99.946
W RS-05.lcd	Metronidazo	W RS-05	7.344	3008880	341060	1.232	14627	100.000
W RS-06.lcd	Metronidazo	W RS-06	7.344	3008333	341354	1.232	14644	100.075
Average			7.343	3006543	341515	1.233	14609	100.001
%RSD			0.017	0.087	0.139	0.043	0.261	0.064

Run time: 15 minutes

4.9.2.2.8. Analysis

Three replicate 5µl sample solutions were injected automatically to the HPLC. Then, the percentage of the labeled amount of Metronidazole benzoate in the brands was calculated by the formula:

$$\text{Result} = (\text{ru}/\text{rs}) \times \left(\frac{\text{Cs}}{\text{Cu}}\right) \times 100\% \text{ (USP, 2015)}$$

Where

$\text{ru} = \text{peak response from the sample solution}$

$\text{rs} = \text{peak response from the standard solution}$

$\text{Cs} = \text{concentration of Metronidazole in the standard solution}$

$\text{Cu} = \text{nominal concentration of Metronidazolein the sample solution(mg/ml)}$

Acceptance criteria: 90.0% -110.0 % (USP, 2015).

4.9.2.3. Microbial limit test

4.9.2.3.1. Total aerobic microbial count (TAMC)

4.9.2.3.1.1. Sample preparation

First, all the seven brands of Metronidazole benzoate oral suspension was visually checked for any irregularity and stored at room temperature. Then, sample number was assigned to the brands based on their expiry. The outer surfaces of bottles were cleansed with 70% isopropyl alcohol and placed in laminar air flow room and allowed to air dry. The exterior of each

bottle was disinfected with 70% Isopropyl alcohol and shacked to maximize microbial dispersment and transferred to working room. Then, each unit container was aseptically opened in a working room and 1ml of the sample was mixed with 10ml of distilled water and filtered through 0.45µm membrane filter. The membrane filter was rinsed with five 10ml of distilled water. Finally, 100 ml of TSA was transferred to the membrane filter and incubated at 32°C for 3days.

4.9.2.3.1.2. Negative control

As negative control, 100 ml tryptone soya agar, TSA, were incubated at 32°C for 3 days.

4.9.2.3.1.3. Examination

The samples under the test and the negative control were examined visually for the number of colony forming units on daily basis.

Acceptance criteria: There must be no growth of micro-organism in the negative control and TAMC of the sample should be <1000 CFU/ml.

4.9.2.4. Specific gravity

First, pycnometer was cleaned and dried. Then, the tare weight of the pycnometer was determined by weighing it in balance and was found to be 11.6369 gm. Then, the pycnometer was filled with water. The water filled pycnometer was weighed and was found to be 22.8268gm. Then, the weight of water was determined by subtracting the weight of empty pycnometer from the weight of water filled pycnometer and was found to be 11.1899gm. Then, the pycnometer was filled with the samples and the respective weight of the sample filled pycnometer was determined. Then, the weight of each sample was determined by subtracting the tare weight of the pycnometer from respective weight of sample filled pycnometer. Finally, the specific gravity of each sample was obtained by dividing the weight of sample by weight of water.

Acceptance criteria: The ideal specific gravity value is 1.

4.9.2.5. pH Test

First, the pH sensor was rinsed with water and then with a few portions of the sample. Then, the pH sensor was immersed in to the test sample and sufficient time was allowed for stabilization of the pH measurement. Then, pH value was recorded for each sample.

Acceptance criteria: 5-6.5 (BP, 2013).

4.9.3. Metronidazole injection

4.9.3.1. Identification test

4.9.3.1.1. Preparation of sample solution

First, 20 ml of Metronidazole injection and 9g NaCl was collected in separatory funnel and shacked for 5 minutes (BP, 2013). Then, 20 ml of acetone was added to it and the mixture was allowed to separate. Then, the upper layer was evaporated to dryness in an evaporating dish and 10 mg of the sample was taken in KBr plate to FTIR instrument for the identification test (BP, 2013).

4.9.3.1.2. Analysis

First, 10 mg of Metronidazole ICRS was placed on KBr plate and taken to the FTIR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1}(BP, 2013). Then, 10mg of Metronidazole sample was taken with KBr plate to IR instrument and its IR absorption spectrum was measured in a wave number range of 400cm^{-1} to 4000 cm^{-1} (BP, 2013).

Acceptance criteria: The IR absorption spectrum of the standard and the samples under study should coincide.

4.9.3.2. Assay test

4.9.3.2.1. Preparation of mobile phase

Mobile phase was prepared by dissolving 0.68 g of monobasic potassium phosphate in 930 ml of water and 70 ml of methanol. Then, the pH of the mobile phase was adjusted to pH 4 ± 0.5 with 1M phosphoric acid (USP, 2015).

4.9.3.2.2. Preparation of standard stock solution

Standard stock solution of strength 1mg/ml was prepared by dissolving 10 mg of Metronidazole ICRS in 10 ml of methanol (USP, 2015).

4.9.3.2.3. Preparation of standard solution

Standard solution was prepared by mixing 5 ml of standard stock solution and 5ml of water in 25 ml of volumetric flask and diluting the resulting solution with the mobile phase to a volume resulting in formation of standard solution of strength, 0.2mg/ml.

4.9.3.2.4. Preparation of sample stock solution

Samples stock solution of strength 1 mg/ml was prepared by dissolving 20 ml of Metronidazole injection, the volume that contains 100 mg of Metronidazole, in 100 ml of water (USP, 2015).

4.9.3.2.5. Preparation of sample solution

To prepare sample solution, 5ml of sample stock solution and 5ml of water was combined in 25ml volumetric flask and diluting with a mobile phase to a volume resulting in formation of sample solution of strength, 0.2mg/ml (USP, 2015).

4.9.3.2.6. Chromatographic system

An Agilent HPLC system (a column: 4.6mm x 25cm; with 5µm internal packaging L1, detector: PDA detector (λmax= 320 nm)) was used (USP, 2015).

Run time: 25 minutes.

4.9.3.2.7. System Suitability

Five replicate injections of 20µl of standard preparations were injected automatically to the HPLC (USP, 2015).

Acceptance criteria: Relative standard deviation for five replicate injections should be NMT 2% and a tailing factor NMT 2 **(Table 6).**

Table 6: System suitability test result of Metronidazole injections (n=5).

<< PDA >>
ID#1 Compound Name: Metronidazole

Title	ample Nam	Sample ID	Ret. Time	Area	Height	Tailing Factor	er of Theoretical Plate	Conc.
C RS-01.lcd	Metronidazo	C RS-01	12.620	6187004	410727	1.088	15763	100.000
C RS-02.lcd	Metronidazo	C RS-02	12.526	6183427	412819	1.091	15712	99.971
C RS-03.lcd	Metronidazo	C RS-03	12.511	6181185	410893	1.096	15484	99.957
C RS-04.lcd	Metronidazo	C RS-04	12.631	6186765	405885	1.102	15430	100.035
C RS-05.lcd	Metronidazo	C RS-05	12.684	6183382	404531	1.107	15468	99.984
Average			12.595	6184353	408971	1.097	15571	99.989
%RSD			0.586	0.040	0.872	0.689	0.988	0.030

4.9.3.2.8. Analysis

Three replicate 20µl sample solution were injected automatically to the HPLC. Then the percentage of the labeled amount of Metronidazole benzoate in the brands was calculated by the formula:

$$\text{Result} = (\text{ru}/\text{rs}) \times (\frac{\text{Cs}}{\text{cu}}) \times 100\% \text{ (USP, 2015)}$$

Where

$\text{ru} = \text{peak response from the sample solution}$

$\text{rs} = \text{peak response from the standard solution}$

$\text{Cs} = \text{concentration of USP Metronidazole RS instandard solution}(\frac{\text{mg}}{\text{ml}})$ $\text{Cu} = \text{nominal concentration of the sample solution}(\text{mg}/\text{ml})$

Acceptance criteria: 90.0%-110.0% (USP, 2015).

4.9.3.3. pH Test

First, the pH sensor was rinsed with water and then with a few portions of the sample. Then, the pH sensor was immersed in to the test sample and sufficient time was allowed for stabilization of the pH measurement. Then, pH value was recorded for each sample.

Acceptance criteria: 4.5-6 (BP, 2013).

4.9.3.4. Sterility test

4.9.3.4.1. Sample preparation

First, the bottles were examined visually for container closure integrity, for the presence of any foreign matter and other defects present in the product. Then, sample identification number was assigned based on the expiry date of the samples for the traceability of the samples and the exterior of all product primary containers was cleansed with 70% IPA and allowed to completely dry under UV ray for 24 hours in laminar air flow room. Then, the samples were taken to the working area and all the contents of the bottles were aseptically filtered through a two 0.45µm membrane filters. The filters was rinsed with five 10 ml of distilled water. Finally, to each membrane filter, each 100 ml of tryptone soya broth and thioglycolate medium was transferred and incubated for 14 days at 22°C and 32 °C respectively.

4.9.3.4.2. Negative Control

Each 100 ml of tryptone soya broth and thioglycolate medium were incubated for 14 days at 22°C and 32°C respectively for negative control.

4.9.3.4.3. Examination

The samples and the negative control were inspected daily for growth of micro-organisms.

Acceptance criteria: There should be no growth of micro-organisms in the negative controls and the samples.

4.9.3.5. Endotoxin test

4.9.3.5.1. Preparation of Endotoxin standard stock solution

Standard Endotoxin stock solution was prepared by mixing 5ml of water for BET and 5 Endotoxin units.

4.9.3.5.2. Preparation of standard Endotoxin solution

Standard Endotoxin solution was prepared by one to thousand serial dilution of Endotoxin standard stock solution.

4.9.3.5.3. Preparation of LAL solution

LAL solution was prepared by mixing 1.2ml of water for bacterial Endotoxin test (BET) with LAL reagent.

4.9.3.5.4. Preparation of sample stock solution

First, maximum valid dilution for the samples under the test was determined by formula:

$$\mathrm{MVD} = \frac{\mathrm{EL} \times \mathrm{C}}{\lambda}$$

Where

MVD = Maximum valid dilution

EL = Endotoxin limit which 0.35

C= Concentration of the samples which 5mg/ml

λ= Lysate sensitivity which 0.25

MVD= $\frac{0.35\times5\text{mg/ml}}{0.25} = 7$

Then, sample stock solution was prepared by mixing 1ml of sample solution and 6ml of water for BET in volumetric flask and shacked for 3 minutes.

4.9.3.5.5. Preparation of sample solution

The sample solution was prepared by mixing 0.1 ml of the sample stock solution and 0.1 ml of LAL solution.

4.9.3.5.6. Positive control

Two replicate positive controls were prepared by mixing 0.1ml standard Endotoxin solution and 0.1ml LAL in a test tube.

4.9.3.5.7. Negative control

Two replicate negative controls were prepared by mixing 0.1ml of LAL and 0.1ml of water for injection in a test tube.

4.9.3.5.8. Storage of the preparations

Immediately after preparation, all the preparations were incubated at 37°C for 1 hour in an incubator.

4.9.3.5.9. Examination

At the end of 1hr of incubation, each test tube was taken in turn directly from the incubator and inverted through 180° in one turn smooth motion and its result was recorded. The result was recorded positive if intact cloth that does not dissolve in 180° smooth rotation was formed and if not it was recorded negative.

Acceptance criteria: The replicate results of negative and positive control should be negative and positive respectively and the Endotoxin concentration of the sample should be <0.35Eu/ml (USP, 2015).

4.10. Quality assurance

Sample collectors (i.e.mystery shoppers) were trained for 1 day. The training was given on allocation of pharmacies and drug stores for sample collection and how to purchase enough samples to allow for quality assessment (WHO, 2015). Two mystery shoppers per site were assigned for sample collection, one to purchase the drug and the other to handle and file the data of the purchased samples. The manufacturer and brand name, batch numbers, date of manufacture, expiry dates, place of collection and type of the drug outlet from which the samples were purchased was recorded in the format immediately after leaving the pharmacies and drug stores from which the respective brands are purchased. The quality of data was checked by investigator. Then, the samples were brought to EPHARM quality control laboratory next day to the day of completion of sample collection for analysis. Next to the day of completion of sample collection (i.e. on May 23, 2018), the samples was taken for laboratory analysis to EPHARM quality control laboratory in bag that protects the samples from direct sunlight and stored in conditions recommended on the respective the label claim of the brands in the EPHARM quality control laboratory sample storage section. To ensure reliability of results, calibrated and validated equipments was used for all procedures and relevant standard operating procedures (SOPs) was followed for all tests during the laboratory analysis of the samples. All the chemicals and reagents used were of analytical grade and prequalified. The respective test result was carefully written and handled.

4.11. Data analysis

The quality of the samples were assessed based up on a method specified in BP 2013 and USP 2015 and the test results were compared with respective official specifications of BP, 2013 and USP, 2015. The assay result of all the fourteen brands of Metronidazole and dissolution test result of the three brands of Metronidazole capsule was entered to statistical package for social sciences software version 24.0 for windows. Then, one way analysis of variance (ANOVA) was performed using Tukey test to determine whether there exists significant difference in assay and dissolution test results within and among the brands of the respective dosage forms ($p<0.05$).

4.12. Ethical approval

The study was reviewed and approved by the ethical review committee of Jimma University, Institute of Health**.**

4.13. Dissemination plan

The findings of this study shall be communicated to EFMHACA, EFMHACA Jimma branch. Then, the finding of the study shall be published on journal whose publication is primarily focused on researches involving pharmaceuticals quality.

4.14. Variable definitions

Assay: Content of active pharmaceutical ingredient.

Biopharmaceutical classification system class I (BCSI) drugs: Drugs that have high permeability and high solubility and rate limiting step for absorption to systemic circulation is dissolution of the drug.

Contamination: The undesired introduction microbes to a pharmaceutical dosage form above tolerance limit tolerable in official compendia's.

cGMP: Current good manufacturing practice that involves updating technology in GMP.

Dissolution: Rate and degree of release of active ingredients in liquid medium.

Good dispensing practice: Delivery of the correct medicine to the right patient, in the required dosage and quantities with clear medicine information counseling and appropriate follow up.

Good distribution practice: Distribution of pharmaceuticals according to the principles of GMP and good storage practice (GSP) that maintains the stability of the drug thought its shelf life.

GMP: Manufacturing practice that enables the manufacture of pharmaceutical products continuously and consistently in quality standard appropriate for intended use.

Identity: Presence of specified active ingredient.

Stability of medicines: The ability of the medicines to maintain the physical, chemical, and microbial properties during the time of storage and usage by the patient.

Poor quality: Drugs that fail to meet quality specification set for them.

Specification: Set of criteria to which a drug product should conform to be considered acceptable for its intended use.

5. RESULTS

5.1. Identification test result

The identification test for the fourteen brands was performed according to a method indicated in BP 2013 for identification of the drug. The merged FTIR spectrum of the reference standard and the samples is illustrated below in figure 2, 3 and 4. As indicated on the figures, the IR spectrums of the standard and the samples are coinciding, indicating the standard and the fourteen brands of Metronidazole analyzed on the present study have similar IR spectrum absorption, which in turn shows identity of API of interest in the samples. Accordingly, all the brands passed the test for identification test.

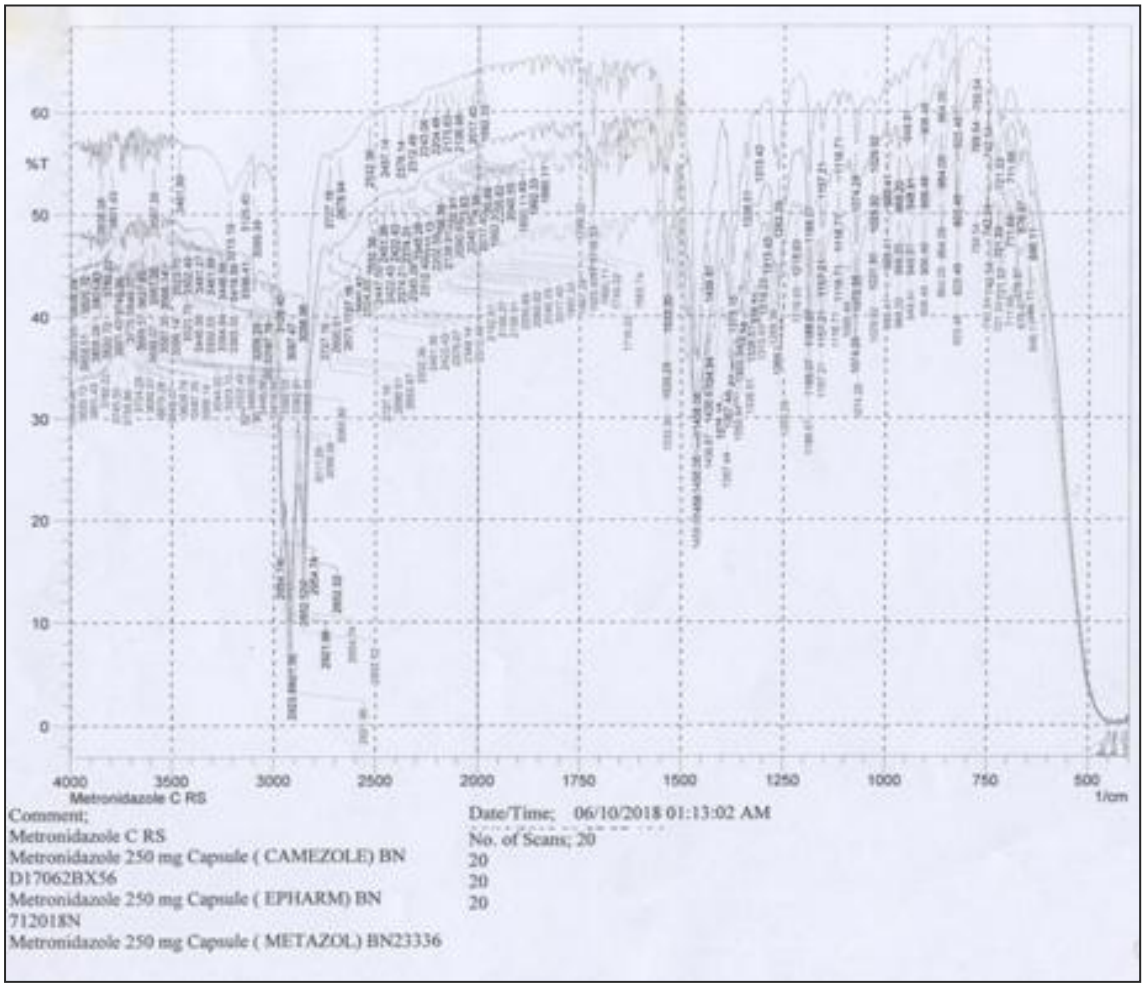

Figure 2: Merged FTIR spectrum of three brands Metronidazole capsules and Metronidazole ICRS.

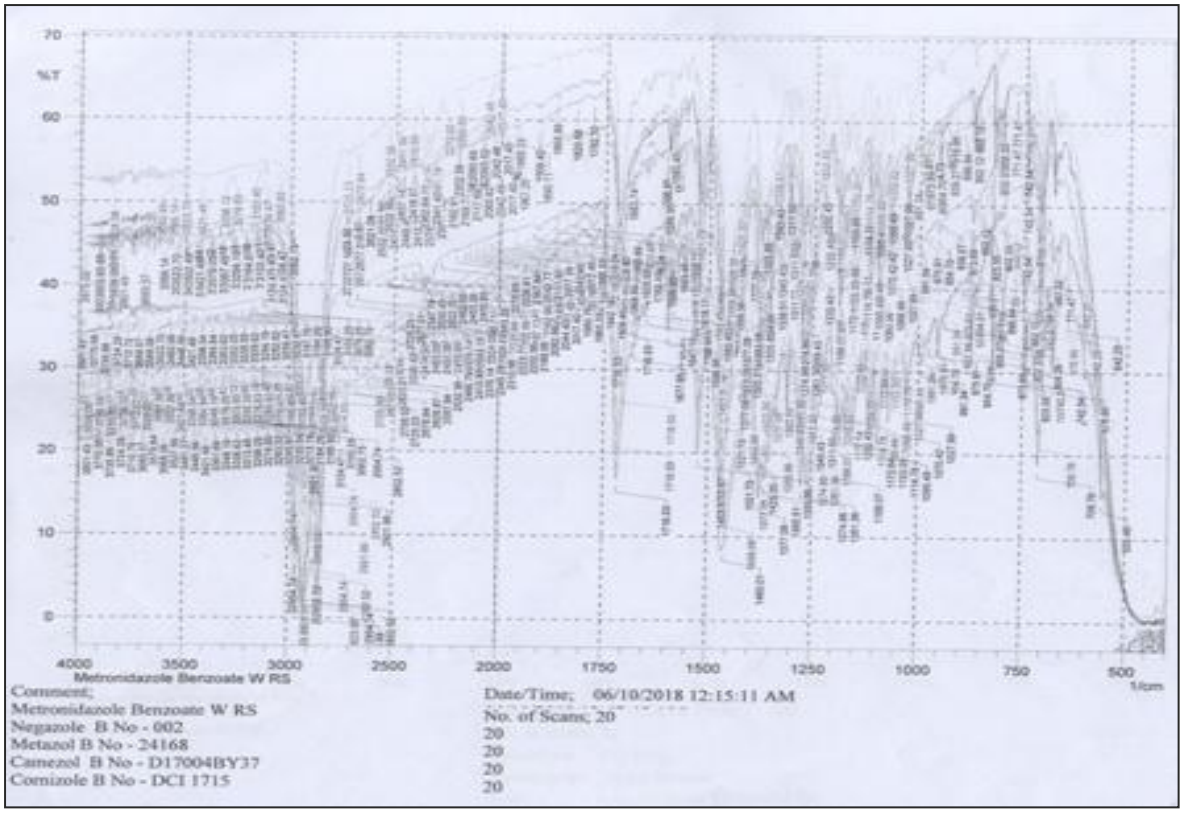

Figure 3: Merged FTIR spectrum of Metronidazole benzoate oral suspensions and Metronidazole benzoate RS.

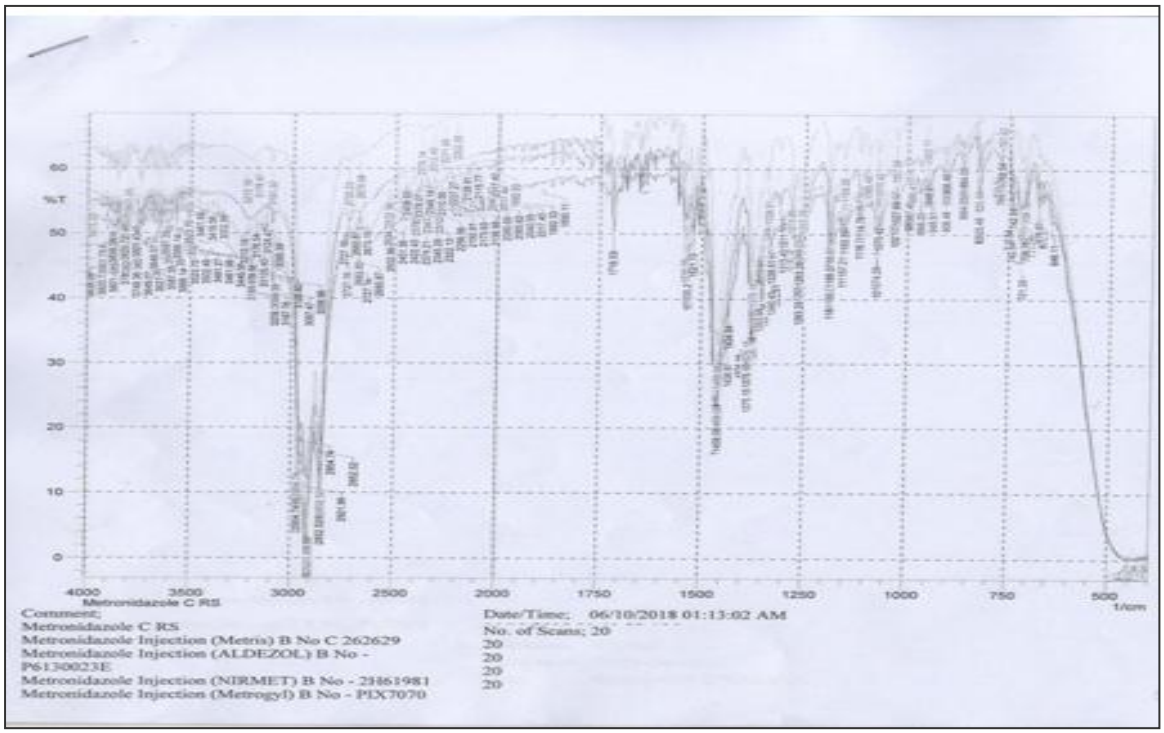

Figure 4: Merged FTIR spectrum of four brands Metronidazole injections and Metronidazole ICRS.

5.2. Weight uniformity test result of Metronidazole capsules

Weight uniformity test was done using a method specified in USP 2015. According to USP 2015 specification, the requirements for dosage uniformity are met if the acceptance value (AV) of the first 10 dosage units is less than or equal to L1, which 15. Weight uniformity test result of Metronidazole capsule brands is shown in table 7. Thus, according to the results, all the three brands of Metronidazole capsules passed a weight uniformity test.

Table 7: Weight uniformity test result of randomly selected Metronidazole capsules (n=10).

Brand Name	av.xi (%)	sd	% RSD	M (%)	Acceptance value (%)	USP 2015 limit
Metronidazole(generic)	107.55	1.32	1.23	101.5	9.218	L1<15
Camezol	102.71	2.63	2.56	101.5	7.522	L1<15
Metazol	104.04	2.2	2.12	101.5	7.82	L1<15

5.3. Assay of Metronidazole capsules, Metronidazole benzoate oral suspensions and Metronidazole injections

The products were assayed according to the method outlined in USP 2015, in which it is indicated that the products assay (i.e. drug content) should lie in a range of 90% to110% of label claim. Thus, as per the results all the fourteen brands of Metronidazole assessed for quality passed the assay test specification. The results for the label claim of the fourteen brands of Metronidazole (three Metronidazole capsule, seven Metronidazole benzoate oral suspension and four brands of Metronidazole injection) analyzed in current is shown in table 8 and the HPLC chromatogram of reference standards and selected samples is indicated on figure 5, 7, 9 and 6, 8, 10 respectively.

5.3.1. Assay of Metronidazole capsules

All brands of Metronidazole capsules studied passed USP 2015 specification for assay of the product. The highest percentage of drug content was obtained for Metronidazole (generic), 107.81% followed by Metazol and Camezol, 104.34% and 102.96% respectively. Statistical comparison of drug contents at 95% confidence interval indicates that there exists significant difference in drug content within and among the three brands of Metronidazole capsules ($p<0.05$).

5.3.2. Assay of Metronidazole benzoate oral suspensions

All brands of Metronidazole benzoate oral suspension passed the test for assay of active pharmaceutical ingredients as per USP 2015 specification. The highest percentage of drug content was obtained for Metrolag, 105.56%, while the least content for Mizel, 93.12%. Statistical comparison of drug contents at 95% confidence interval indicates that there is significant difference in drug content within and among the seven brands of Metronidazole benzoate oral suspensions ($p<0.05$).

5.3.3. Assay of Metronidazole Injections

All brands of Metronidazole injection analyzed for quality passed USP 2015 specification for assay of the product. The highest percentage of drug content was obtained for Nirmet, 101.03%, while the least content was obtained for Aldezol, 96.98%. Statistical comparison of drug contents at 95% confidence interval indicates that there is significant difference in drug content within and among the four brands of Metronidazole injections ($p<0.05$).

Table 8: Assay result of Metronidazole capsules, injections and Metronidazole benzoate oral suspensions (n=3).

Product	Brand Name	Assay Result (%)	±RSD (%)	USP 2015 limit
Metronidazole Capsule	Metronidazole(Generic)	107.81	0.113	90-110%
	Camezol	102.96	0.229	90-110%
	Metazol	104.34	0.138	90-110%
Metonidazole benzoate suspension	Camezol	102.06	0.102	90-110%
	Cornizole	101.23	0.233	90-110%
	Metazol	93.94	0.042	90-110%
	Metrolag	105.56	0.05	90-110%
	Metrogyl	104	0.110	90-110%
	Mizel	93.12	0.089	90-110%
	Negazole	101.7	0.051	90-110%
Metronidazole Injection	Aldezol	96.98	0.018	90-110%
	Metrogyl	99.63	0.003	90-110%
	Metris	100.3	0.021	90-110%
	Nirmet	101.03	0.009	90-110%

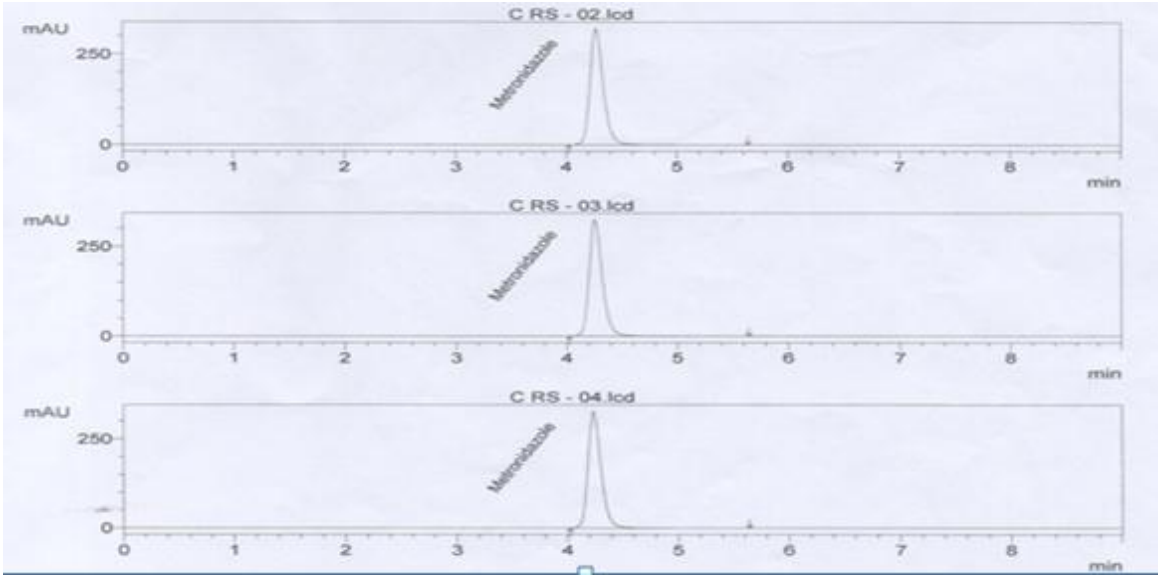

Figure 5: HPLC chromatogram of Metronidazole ICRS for Metronidazole capsules.

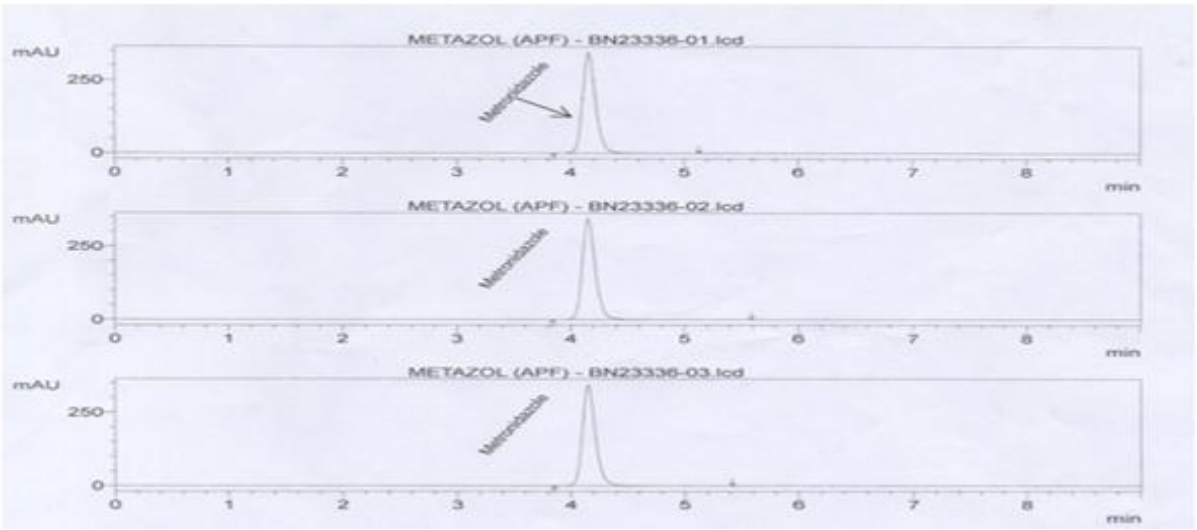

Figure 6: HPLC chromatogram of Metazol sample.

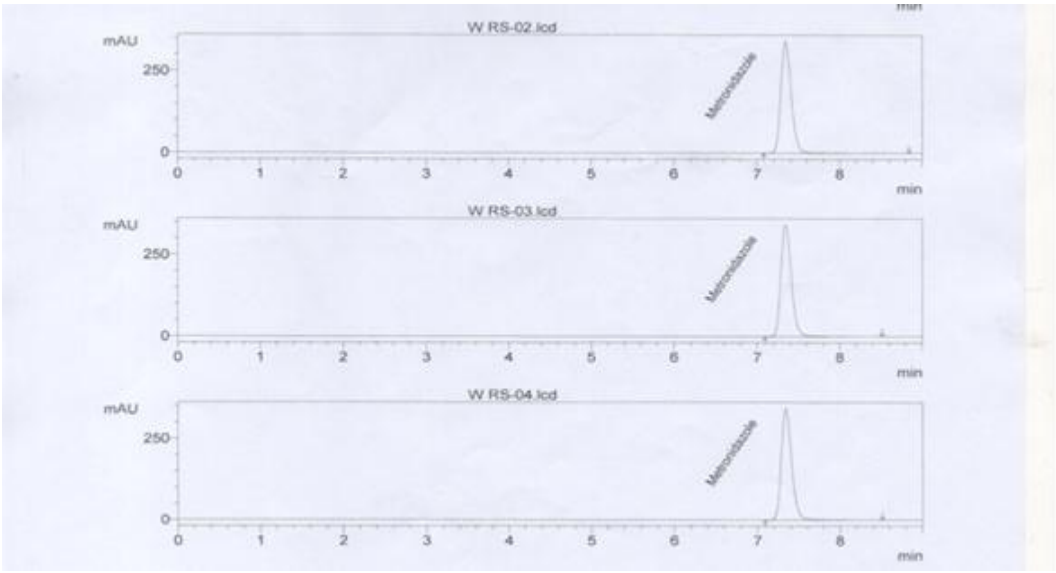

Figure 7: HPLC chromatogram of Metronidazole benzoate reference standard.

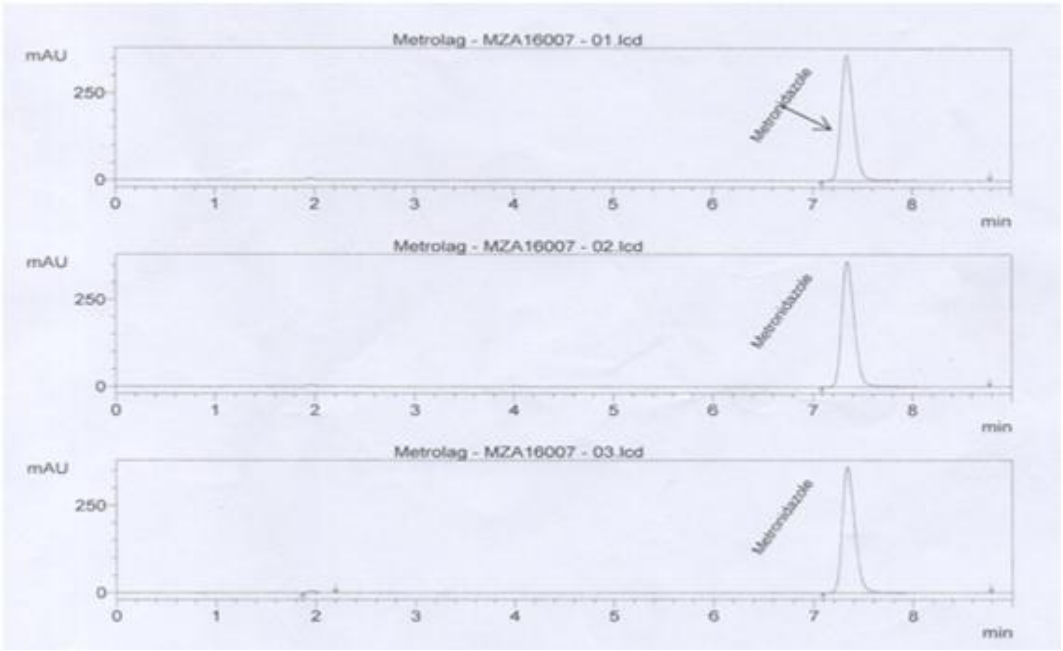

Figure 8: HPLC chromatogram Metrolag.

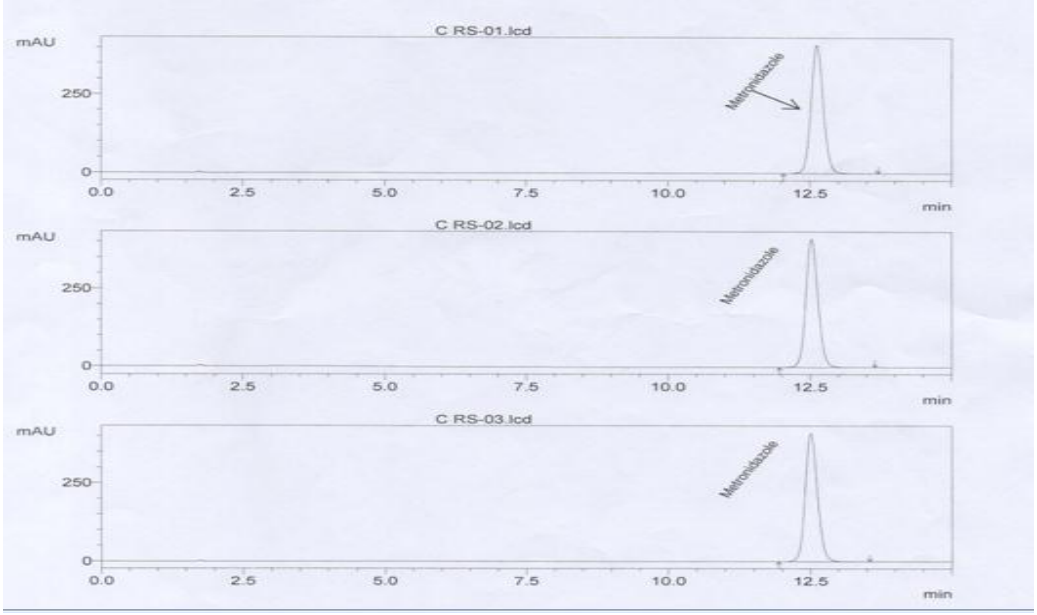

Figure 9: HPLC chromatogram of Metronidazole ICRS for Metronidazole injections.

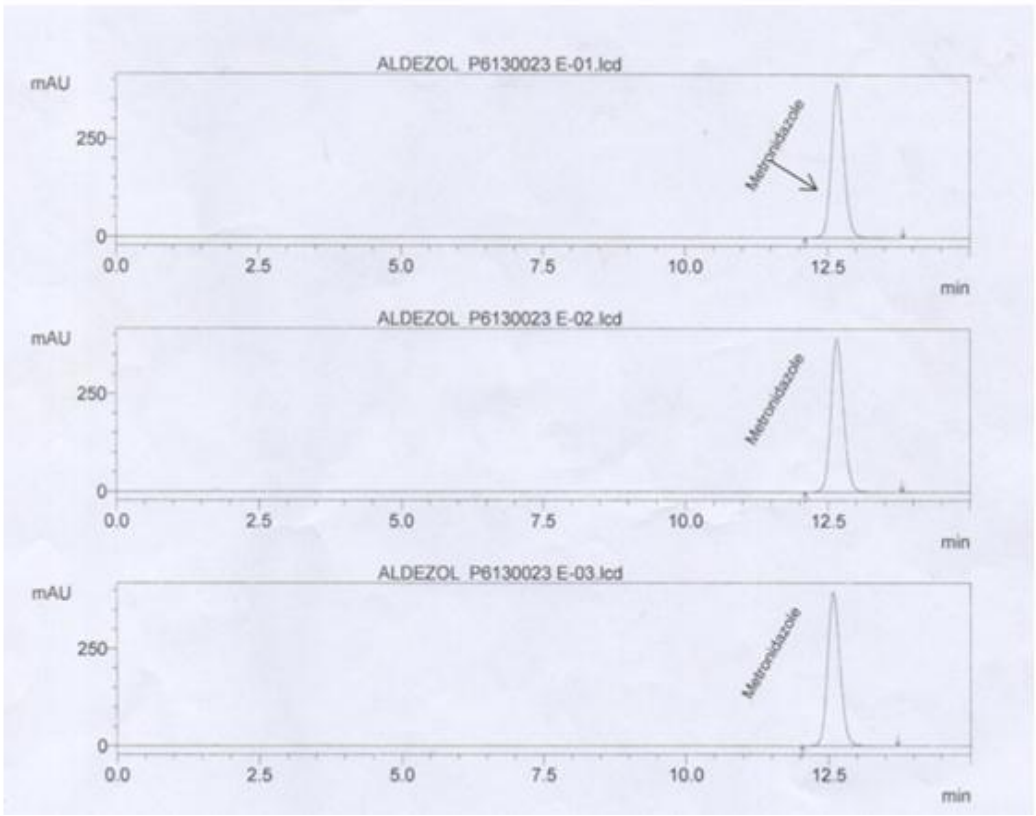

Figure 10: HPLC chromatogram of Aldezol.

5.4. Dissolution test result of Metronidazole capsules

The dissolution test for the Metronidazole capsules was performed using a method specified in USP 2015, in which it is indicated that not less than 85% of the active ingredient should be released in 30 minutes. In the current study, all the three brands of Metronidazole capsules studied released more than 85% of API within 30 minutes. The highest percentage of dissolution was obtained for Camezol, 106.61%, followed by Metazol and generic Metronidazole, 101.76% and 99.71% respectively. Statistical comparison of dissolution (release of API) at 95% confidence interval revealed that there exists significant difference in drug release within and among the three brands of Metronidazole capsules ($p<0.05$). The time dependent dissolution result and dissolution profile of the brands is shown in Table 9 and figure 11 respectively.

Table 9: Result of time dependent dissolution of randomly selected Metronidazole 250 mg capsules (n=6).

Sampling time (min)	% drug released (mean ± RSD; n = 6)		
	Metronidazole (generic)	Metazol	Camezol
10	93.68 ± 2.25	94.55 ± 2.55	100.67 ± 1.11
15	97.23 ± 1.04	98.47 ± 1.3	102.04 ± 1.18
20	98.26 ± 1.19	100.96 ± 0.97	103.76 ± 1.16
30	99.71 ± 1.19	101.76 ± 1	106.61 ± 1.16
45	100.1 ± 1.507	101.86 ± 0.64	106.86 ± 1.4
60	99.81 ± 0.788	102.80 ± 1.41	106.69 ± 1.51

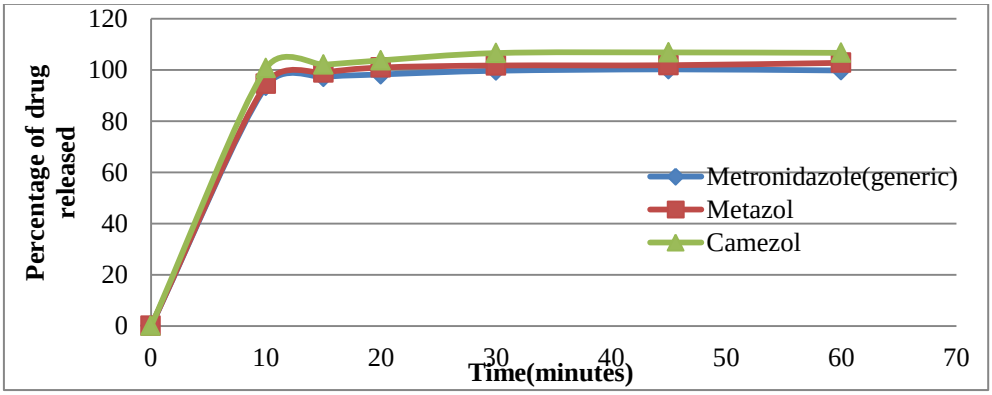

Figure 11: Time dependent dissolution profile of three brands of Metronidazole capsules (n=6).

5.5. pH test result of Metronidazole benzoate oral suspensions

The pH of all the brands of Metronidazole suspensions analyzed in the present study is within a tolerance range of BP 2013 **(Table 10).** For Metronidazole suspensions to be stable and hence to be therapeutically effective, its pH should remain in the specified limit throughout the shelf life.

Table 10: pH test result of Metronidazole benzoate oral suspensions.

Brand name	pH value	pH Range (as per BP 2013)
Camezol	5.50	5-6.5
Cornizole	5.29	5-6.5
Metazol	5.6	5-6.5
Metrogyl	5.43	5-6.5
Metrolag	5.91	5-6.5
Mizel	5.86	5-6.5
Negazole	6.05	5-6.5

5.6. pH test result of Metronidazole injections

The pH of all brands of Metronidazole injection analyzed on the current study is within BP 2013 tolerance range for pH of the product, 4.5-6 **(Table 11).**

Table 11: pH test result of Metronidazole injections.

Brand name	pH value	pH Range (as per BP 2013)
Aldezol	5.28	4.5-6
Metris	5.28	4.5-6
Metrogyl	5.53	4.5-6
Nirmet	5.7	4.5-6

5.7. Specific gravity test result of Metronidazole benzoate oral suspensions

For suspensions to have good dispersion of the ingredients, the specific gravity of the suspension should be around one. As indicated below on table 12, all Metronidazole benzoate oral suspensions have a specific gravity value more than one which could cause sedimentation of the ingredients suspension which results in instability and uneven distribution of the API of the suspension.

Table 12: Specific gravity test result of Metronidazole benzoate oral suspensions.

Brand name	Total weight	Weight of the sample	Specific gravity
Camezol	24.7787	3.1418	1.1744
Cornizole	25.3586	13.7217	1.2263
Metazol	24.8812	13.2443	1.1836
Metrolag	24.9294	13.2925	1.1879
Metrogyl	25.8062	14.1698	1.2663
Mizel	25.1408	13.4539	1.2023
Negazole	25.1206	13.4837	1.2050

5.8. Total aerobic microbial count (TAMC) test result of Metronidazole benzoate oral suspensions.

The suspensions were tested for total aerobic microbial count according to a method specified in USP 2015. Non-sterile pharmaceutical products total aerobic microbial count should be less than 1000 CFU/ml after 3-5 days of incubation in the culture. Thus, all the seven brands of Metronidazole benzoate suspension analyzed passed TAMC test**.** The results of total aerobic microbial count (TAMC) and its negative control are shown in table 13 and 14.

Table 13: Total aerobic microbial count (TAMC) test result of Metronidazole benzoate oral suspensions.

Brand name	TAMC (CFU/ml)	Limit
Camezol	20	<1000 CFU/ml
Cornizole	10	<1000 CFU/ml
Metazol	<10	<1000 CFU/ml
Metrogyl	20	<1000 CFU/ml
Metrolag	10	<1000 CFU/ml
Mizel	<10	<1000 CFU/ml
Negazole	<10	<1000 CFU/ml

Table 14: Metronidazole benzoate oral suspensions total aerobic microbial count (TAMC) test negative control test result.

Negative control	Result
100 ml Tryptone soya agar	Negative (-ve)

5.9. Sterility test result of Metronidazole injections

Sterility test result of the samples is indicated in table 15 and the result of negative control in table 16. The samples were analyzed for sterility as per a method indicated in USP 2015. Two brands (i.e. Aldezol and Metris) failed sterility test from the four brands of Metronidazole injections included in the study and hence of poor quality.

Table 15: Sterility test result of Metronidazole injections.

Product name	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aldezol	-ve	-ve	-ve	-ve	-ve	-ve	+ve							
Metris	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve				
Metrogyl	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nirmet	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 16: Metronidazole injections sterility test negative control test result.

Negative control	Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Tryptone soya broth	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Thioglycolate medium	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Figure 12: Image of Metronidazole sterility test.

5.10. LAL test result of Metronidazole injections

The products were tested for bacterial Endotoxin test according to a method specified in USP 2015. LAL test result of Metronidazole injections, negative and positive control are shown in table 17, 18 and 19. As indicated on the Table 16, all the four brands of Metronidazole injections passed a test for Endotoxin.

Table 17: LAL test result of Metronidazole injections (n=1).

Brand name	Endotoxin concentration	USP 2015 limit
Aldezol	<0.25Eu/ml	<0.35Eu/ml
Metris	<0.25Eu/ml	<0.35Eu/ml
Metrogyl	<0.25Eu/ml	<0.35Eu/ml
Nirmet	<0.25Eu/ml	<0.35Eu/ml

Table 18: Metronidazole injections LAL test negative control test result (n=2).

Negative control	Result
0.1 ml of LAL +0.1 ml of water for BET	Negative (-ve)

Table 19: Metronidazole injections LAL test positive control test result (n=2).

Positive control	Result
0.1ml standard Endotoxin solution + 0.1 ml water for BET	Positive (+ve)

5.11. Comparison of price of the fourteen brands Metronidazole analyzed for quality

All the fourteen brands prices were converted to US dollars according to the exchange rate of the purchase date. The price and retail outlets from which the all the fourteen brands of Metronidazole analyzed on the present study is shown in table 20. The unit price of Metronidazole (generic) is 22% lower than the price of metazol. The cost of Metrolag suspension, 6.96 USD, is 9.87 times the average cost of the six brands of Metronidazole suspensions, which is 0.705 USD. The cost Aldezol is 48% lower than the cost of Nirmet and Metrogyl. The average price of all the fourteen brands is 0.97 USD.

Table 20: Price and retail outlet Information of the fourteen brands Metronidazole analyzed for quality.

Product	Brand Name	Retail Outlet	Unit Price*(USD)
Metronidazole Capsule	Metronidazole(Generic)	Pharmacy	0.18
	Camezol	Pharmacy	0.18
	Metazol	Drug store	0.22
Metonidazole benzoate suspension	Camezol	Pharmacy	0.6
	Cornizole	Pharmacy	0.77
	Metazol	Drug store	0.66
	Metrolag	Drug store	6.96
	Metrogyl	Drug store	0.77
	Mizel	Drug store	0.66
	Negazole	Pharmacy	0.77
Metronidazole Injection	Aldezol	Pharmacy	0.37
	Metris	Drug store	0.33
	Metrogyl	Drug store	0.55
	Nirmet	Pharmacy	0.55

Where,

Unit Price*: Price 1strip for Metronidazole capsule

Price of 1bottle for Metronidazole benzoate oral suspension and

Price of 1 bottle of Metronidazole injection

6. DISCUSSION

For drugs to bring intended therapeutic effect, the drugs should contain the API of interest that is responsible for the pharmacological effect. In the current study, all the fourteen brands of Metronidazole assessed for quality passed BP 2013 specification for identity of the respective dosage forms. Identification tests performed on the drug in various countries demonstrated mixed results. As in the present study, all the brands analyzed passed an identity test in a study done at Addis Ababa, Ethiopia (Kahaliw and Ashenafi, 2013), Zaria, Nigeria (Musa et al., 2011) and Mongolia (Khurelbat et al., 2014). In contrary to this study finding, in a study done in Nigeria on 581 drugs, in which the 36 of the samples are Metronidazole tablets and 5 of samples are Metronidazole suspensions, all the Metronidazole suspensions included in the study failed identification test (Taylor et al., 2001). The possible reason may be the samples were falsified.

Treatment with medicines with no active ingredient by failing to suppress infection, leads to build up of pathogens, progression of underlying disease and engenders the development of drug resistance (Newton et al., 2017 ; Pisani, 2015).

Consistent dose of the API should be maintained between batches of drug formulations for all patients to receive the correct dose continuously and consistently. In the present study, all the three brands of Metronidazole capsules passed a test for weight uniformity. As is in this study, all brands of Metronidazole analyzed for quality passed weight uniformity in studies done in Eastern Nigeria (Ibezim et al., 2008), Zaria, Nigeria (Musa et al., 2011) and Addis Ababa, Ethiopia (Kahaliw and Ashenafi, 2013). In opposite to this study finding, weight uniformity test failure was reported in a study conducted in Mongolia (Khurelbat et al., 2014). The cause of the discrepancy may be sample size and sample collection technique difference. Unlike this study sample size and sample collection technique, large samples collected by random sampling was analyzed, a method that enables detection of samples quality problems as non-weight uniformity. Variation among dosage units causes patients to receive unnecessary high or low dose which results in increased side effects, mortality and morbidity.

For drugs to be therapeutically effective, the formulation ought to contain the API in officially approved extent that produces desired therapeutic effect. In the current study, the assay of all brands of Metronidazole capsules, Metronidazole injections and Metronidazole benzoate oral suspensions analyzed were within USP 2015 specification limit for the assay of the respective dosage forms. In concordance with this study finding, all brands analyzed passed a test for assay of API in a study done in Zaria, Nigeria (Musa H et al., 2011), Lagos, Nigeria (Ilomuanya et al., 2015) and Addis Ababa, Ethiopia (Kahaliw and Ashenafi, 2013). However, **s**tatistical comparison of assay of respective brands of the respective dosage forms at 95% confidence interval indicates that there exists significant difference in assay within and among the brands of the respective dosage forms ($p<0.05$). In discrepancy with this study finding with respect to assay, failure to comply with assay specification limit was reported in similar in-vitro quality assessment studies conducted on the drug in Eastern Nigeria (Ibezim et al., 2008), Myanmar (WHO, 1999), Bangladesh (Ahmed, 2003) and China (Pan H et al., 2016), where 3 from 10 brands of Metronidazole tablets, 1 from 14 Metronidazole tablets, 6 from 40 brands of Metronidazole (4 from 30 brands of Metronidazole tablets, 2 from 10 brands of Metronidazole suspension) and 41 from 108 Metronidazole failed assay test in the respective studies. The cause for the products to fail assay test on the studies may be the drugs analyzed on respective studies were falsified, as other studies done on the countries indicate prevalence counterfeit drugs that when tested for quality failed quality assessment tests or non-compliance to good practices starting from manufacture to distribution and storage of the products due to weak pharmaceuticals regulatory laws as studies indicate prevalence of such scenario (SPS, 2011 ; Bate et al., 2014).

Treatment of patients with Antibiotics that contain low amount of active pharmaceutical ingredients causes huge negative consequences like drug resistance, treatment failure and increased treatment costs (Wilson, 2011). Substandard quality (i.e. low dose and high dose of Metronidazole) Metronidazole induces drug resistance (Rasoloson et al., 2002 ; van der Wouden, 1997), which in turn causes deleterious negative impacts such as increased morbidity, mortality, treatment cost, hospital stay days and others.

More than 85% of the drug should dissolve within 30 min for BCS class I drugs as Metronidazole, drugs whose absorption is not influenced by solubility, so that dissolution shall not become the rate-limiting step for the absorption of the drug (Rediguieri et al., 2011 ; Lennernas and Abrahamsson, 2005). In the present study, all the three brands of Metronidazole capsules analyzed for quality released > 85% in 30 minutes, which is an acceptable limit of USP 2015. As is in the current study, all brands of Metronidazole assessed for quality passed dissolution test in studies done in Dhaka, Bangladesh (Chowdhury, 2015) and Zaria, Nigeria (Musa et al., 2011). This study finding with regard to dissolution is better than study report of similar quality assessment studies done on the drug in Pakistan(Adil et al., 2016) and Eastern Nigeria (Ibezim et al., 2008), where each 2 brands of Metronidazole failed dissolution test from 13 and 10 brands assessed for quality in the respective studies. The cause for the drug to fail dissolution test on the studies may be lack of adherence to GMP during the manufacturing of the drug.

For BCS class I drugs, gastric emptying controls the absorption rate and different dissolution profiles within acceptance limits have no effect on BA when there is no other problems as GIT disease which affects the absorption of the drug (Manafi et al., 2007 ; Kunde et al., 2015 ; Lennernas and Abrahamsson, 2005). When significant portion of a drug fails dissolution, low dose of API is available for absorption into systemic circulation and hence cannot bring intended therapeutic effect. On the other side, the unabsorbed of portion of the drug causes the respective side effects of the drug (Hetal et al., 2010).

The Microbiological quality of the oral suspensions and injections should be maintained in official specifications limit recommended for the respective route of administration throughout the shelf life of the drug for the dosage form to be stable and therapeutically effective. In the present study, all the seven Metronidazole benzoate oral suspension and four Metronidazole injection brands passed total aerobic microbial count and Endotoxin tests respectively. Two brands of Metronidazole injections failed sterility test from the four brands analyzed for sterility. Our microbiological findings were better than study results of studies done in South Eastern Nigeria (Nwakile et al., 2011), Dar Es Salaam, Tanzania (Mwambete et al., 2009) and Sri Lanka (Sudeshika et al., 2014), where 4 from 17 brands of suspensions (1 from 8 Metronidazole suspensions and 3 from 9 brands of co-trimoxazole suspensions), 3 brands of water for injection from 27 brands (24 brands of quinine sulphate and 3 brands of water for injection) and 1 sample(Lactulose suspension) from five samples(samples of Lactulose, Cephalexin, Amoxicillin, Paracetamole and Salbutamol suspensions) was found to be microbiologically contaminated. The cause for the samples to fail microbiological quality tests may be non-adherence to good storage and transport practice during the storage and transport of the products.

Microbial contamination of liquid dosage preparations causes spoilage, degradation and instability of the product. Subsequent treatment of patients who are already immune-compromised with such microbiologically contaminated products causes increased morbidity and mortality (Amrutha et al., 2017 ; Mwambete et al., 2009 ; Hasegawa et al., 1999 ; Fullerton et al., 2016).

7. CONCLUSION AND RECOMMENDATION

7.1. Conclusion

The result of the current study revealed that there was incidence of poor quality Metronidazole in Jimma town. In the present study, all the fourteen brands of Metronidazole analyzed for quality passed identity and assay test specification of BP 2013 and USP 2015 respectively. However, there exists significant difference in assay within and among the brands of the respective dosage forms ($p<0.05$). All the three brands of Metronidazole capsules passed weight uniformity and dissolution specification of USP 2015. The pH of all the seven brands of Metronidazole benzoate oral suspensions and four brands of Metronidazole injections assessed for quality were within BP 2013 specification range for the respective dosage forms. All the four brands of Metronidazole injections studied passed Endotoxin test except two brands that failed sterility test.

7.2. Recommendation

- **For researchers**

The present quality assessment study result may not indicate the quality status of the drug throughout Jimma town or the country, Ethiopia, for samples analyzed in this study were collected by convenience sampling. Therefore, country wide study with strong study design such as cross-sectional study design based up on random sampling should be conducted to assess the exact quality status of the drug throughout the country.

- **For EFMHACA**

Poor quality drug can be imported to the country any time, may be because of poor co-ordination of police and custom, open borders and other reasons such as corruption and causes deleterious impacts on patient, community, health care system and the government. Beside this, even a drug which is of acceptable quality during the release to market may become substandard because of poor transportation, storage and others and causes deadly effects on patients, community, health care system and the government at large. Therefore, EFMHACA and its branches need to perform post marketing quality assessment studies regularly to determine quality status of the drug on market and take appropriate action to prevent incidence of poor quality medicines.

❖ **For NMRAs of developing countries**

The cause of invasion pharmaceutical market by poor quality drugs is multidimensional and hence is the prevention mechanism (Bate et al., 2014; Kaur et al, 2015 ; SPS, 2011). Developing countries such as Sub-Saharan African countries, the main victims of poor quality pharmaceuticals, are countries who have technical, administrative and financial limitations to ensure quality of pharmaceuticals circulating in their country pharmaceutical market (SPS, 2011; WHO, 2010). Therefore NMRAs of this developing countries need to take appropriate measures, as the following measures, that best suits them to prevent exposure of their citizens to poor quality pharmaceuticals and hence from its consequences.

- Make available quality pharmaceuticals at affordable price to the citizens.
- Strengthening of practice of harmonization for medicines registration.
- Increase awareness of prescribers and the people on generic drug prescription and use.
- Formulate strong national medicines regulation laws and implementing of the laws.
- Purchasing of pharmaceuticals from companies that have built principle of quality to their product manufacturing system as employing of quality by design, process analytical, cGMP in the manufacture of medicines and update their pharmaceuticals manufacturing technology with level of development of technology.
- Purchase pharmaceuticals through prequalified medicine suppliers.
- Increase co-operation of police and custom for control of falsified pharmaceuticals
- Adhere to good practice starting from manufacture, storage, distribution, dispensing and finally during drug use by the patient.
- On the long run the countries need to train professionals that ensure and control quality of pharmaceuticals.

8. LIMITATION OF THE STUDY

Since this study is based upon convenience sampling, the result of the study may not indicate the exact quality status of the drug in the study area.

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ANNEX

Sample collection form

Serial number: _____

Name of location/place where sample was taken:

.....

.....

.....

Address (with telephone and fax number, if applicable):

.....

.....

Date of sampling: .....

Names of people who took samples:

1. .....

2. .....

Product name of the sample: .....

Name of (active) starting material (INN, generic or scientific name)

with dosage strength: .....

Dosage form (tablet, capsule, etc.): .....

Batch/lot number: .....

Date of manufacture:..... Expiry date: .....

Registration or license number (if applicable): .....

Name of the manufacturer: .....

Number of sample unit taken (tablet, capsule, etc):

.....

Brief physical/visual description of sample:

.....

.....

.....

.....

.....

Signature of person(s) taking Signature of representative of the

samples establishment where sample(s) was

taken (optional)

1. .....

.....

2. .....