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An In-Vitro Evaluation Study of the Quality Control of Different Brands of Metronidazole Suspensions Marketed in the South and West of the Republic of Yemen

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ABSTRACT

Background: The Yemeni drug market is open, and most of the companies that manufacture drugs are non- research bases, and consequently, it could be expected that some of those products are substandard. Nine samples of this study were collected from Aden and Hodeida: Four brands were locally, three brands were Arabic, one brand was Indian and 5- one brand was the reference (flagyl^{R}). In addition, the suspensions dosage forms were considered the sparingly soluble and we expected low bioavailability. Label on the packages claimed that all samples contained 200 mg/5 ml. The pharmacological uses of metronidazole are an anti-parasite agent used in the treatment of amoebic and other microbial diseases. **The purpose** of this study was to evaluate the nine brands compared with the reference according to standard specifications. **The method** of analysis was carried out by a spectrophotometer at 278 nm by using 0.1 N Hcl against blank. The method of analysis was valid and achieved reproducibility, accuracy, and linearity. The correlation coefficient was close to 1 (0.994). The relative standard deviation percent (RSD%) was 1.53% at a lower limit. **The results** of the study show that 75% of the brands failed to agree with the Pharmacopoeias specifications in physicochemical tests that were accredited before 1980. **The conclusion** of this study was that six brands out of eight did not agree with the specifications. The investigator advises the authority of health that they must conduct many studies in drug quality and take into consideration the advanced criteria such as bioequivalence and drug stability studies.

INTRODUCTION

Metronidazole benzoate (BM) (Fig. 1) is a benzoate ester resulting from the formal condensation of benzoic acid with hydroxyl group of metronidazole. It has been formulated to hydrolyze in gastrointestinal tract to release therapeutic doses of metronidazole over a period of several hours. Metronidazole is rapidly and almost completely absorbed when given by an oral route with bioavailability (BA) between 80 - 90% and approaching toward 100% [1, 2]. The absorption of metronidazole into the systemic circulation was markedly slower following treatment with benzoylmetronidazole than after dosing with metronidazole, but the pharmacokinetic parameters of elimination were unchanged after administration of equimolar doses of benzoylemetronidazole and metronidazole, and according to this study benzoylmetronidazole was not observed in plasma or urine sampled assayed [3]. And hence, it could be concluded that benzoyl metronidazole is a prodrug for metronidazole that leads to increase the duration period of metronidazole. The molecular formula is $C_{13}H_{13}N_3O_4$, whereas the molecular weight is 275.26 g/mol [4]. Physical properties: a white or slightly yellowish crystalline powder. Practically in soluble in water, freely soluble in dichloromethane R [5] while the melting point ranged 99 – 103 °C and purity [6], and the boiling point and density are 372.3 °C, 1.3 g/ml respectively [4].

According to the Annual Report of the Supreme Board of Drugs and Medical Appliances [7] there are different types of dosage forms such as tablets, suspensions and infusions, and the number of brands in the Yemeni market is 78, 55, and 39 respectively. Moreover, most of the companies that marketed their medicines in the Republic of Yemen (ROY) are non-researches companies [7] that constitute more than 95%.

Metronidazole benzoate suspension is a heterogeneous mixture including solid particles that are satisfactory bulky for sedimentation. The Suspension, a liquid dosage delivery system is a convenient way for to administer insoluble or sparingly soluble drugs to pediatric and geriatric patients that have difficulty in swallowing tablets or capsules. It is employed to mask taste and to control absorption rate of the drug. Moreover, drugs so formulated exhibit higher bioavailability than those in solid dosage delivery systems [8]. However, a major challenge in the formulation of oral suspension is that of physical stability: on storage, the solid insoluble drug separates from the vehicle and settles to the bottom of the container, and readily redisperse by gentle shaking resulting in a homogenous suspension by adjustment a

suitable suspending agent that increases viscosity of the medium, so the particles settle slowly. These suspending agents increase sedimentation volume, ease of redispersibility, enhance pour's ability and prevent compact formation [9]. Furthermore, there are different factors which affect active ingredients for forced degradation. They are Time, Temperature and/or with humidity, photo degradation, pH variation, transport and storage conditions [10]. Defect in formulation or manufacturing process may be responsible for the development of generic drugs of poor-quality spurious substandard drugs counterfeits/ is a modern-day menace, which has been and these medicines may vary from therapeutic failure to the occurrence of serious adverse events and even death [11]. Moreover, Khan and Khar (2015) [12] mentioned that globally, every country is the victim of substandard or spurious drugs, which result in life threatening, financial loss of consumer and manufacturer and loss in trust on the health system. The west and south of Yemen are considered as tropical areas in the summer such as Aden and Hodeida, that are our target for this study [13].

The pharmacological action of metronidazole benzoate suspensions

It has been used for the treatment of infections more than 55 years, and it is still in use in the treatment of amoebiasis, giardiasis, infections during pregnancy, bacterial vaginosis, and prophylaxis against anaerobic infection after bowel surgery, wounds abscess, antibiotic associated colitis against helicobacter pylori and Giardia lamblia that cause travelling diarrhea. However, it is used in combination with other antibiotics and either bismuth compounds or proton pump inhibitors for the treatment of peptic ulcer diseases caused by Helicobacter pylori [14]. Houghton et al, (1982) [3] observed that the values for and times of the highest plasma metronidazole concentration after a single oral dose of benzoylmetronidazole, equivalent to 2 g and 400 mg metronidazole were 17 µg/ml at 5.1 h after dosing and 4.6 µg/ml at 3.2 h after dosing, respectively.

Post licensing marketing surveillance or monitoring involves all activities undertaken to obtain more data and information about the product after it had been granted marketing [15] and check at least five years to ensure the safety, efficacy and quality of new drugs [16]. This type of quality control is completely absent in our country.

The previous studies

Frimpong et al (2018) [17] conducted, quality assessment of some essentials children's medicines in Shanti Region in Ghana, the study included 68 samples of 15 different

manufacturers such as metronidazole benzoate, amoxicillin and co-trimoxazole suspensions. etc. The conclusion of this study was that fifty brands failed (73.53) in uniformity content, pH values and microbial limit. Kahaliw and Ashenef [18] (2013) conducted a study that evaluated 5 brands of metronidazole benzoate suspensions in Ethiopia, the results of this study showed that one brand has accepted viscosity, whereas the other showed lower and high viscosity that lead to a poor quality in the first one , difficult in pouring and faster sedimentation in the other products respectively. Thus, there are satisfactory evidences that the market in the ROY contains substandard, adulterated, smuggled medicines that make us predict worse quality in the Yemeni market. The purpose of this study was to compare the pH values, easily redisperse by gentle shaking, and chemical assays of nine brands of metronidazole benzoate suspensions to ensure a desired level of efficacy, safety and quality.

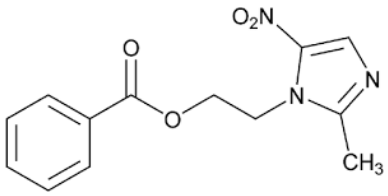

Fig 1. MB chemical structure.

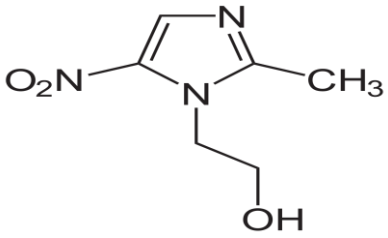

Fig 2. Metronidazole chemical structure.

2. MATERIALS AND METHODS

Collection of samples

Nine different brands of metronidazole suspensions were selected from the targeted areas randomly. The samples were purchased from a retail pharmacy e.g. Aden and Hodeida both of these cities stand by gulf Aden and the Red Sea respectively (Table 1) as fallows 4 locally products, 3 Arabic products, and one brand Indian and one reference product (Flagyl).

Table 1 showing samples of study.

Sample code	Batch no	Sample code	Batch no	Code sample	Batch no
MBS_1 200 mg/5ml	203	MBS_4 200 mg/5ml	09113	MBS_7 200 mg/5ml	09099
MBS_2 200 mg/5ml	09470	MBS_5 200 mg/5ml	186	MBS_8 200 mg/5ml	1729
MBS_3 200 mg/5ml	MY1B9V	MBS_6 200 mg/5ml	082956	MBS_9 200 mg/5ml	1182

MBS: Metronidazole benzoate suspensions

Experimental

Apparatus

A Jasco Japanese spectrophotometer model V – 550, with 1 cm quartz cells were used throughout this research work.

Analytical balance, model: H. D- 77336 kern and Sohn Gmb Ballengin Germany.

pH – meter apparatus, Sartorius AG, Germany.

Materials and reagents

Standard phosphate buffers pH 4: composition citric acid/NaOH solution/Nacl.

Metronidazole benzoate reference (MBR) was kindly supplied by locally manufacturer: Modern Pharma.

Preparation of standard calibration curve

Series of standard solutions with different concentration of MBR e.g. 8, 10, 12, 14 and 16 µg/ml were prepared by dissolving 100 mg of standard metronidazole benzoate in a 100 ml volumetric flask and volume was adjusted by 0.1N HCl (stock solution). Then 0.8, 1, 1.2, 1.4, and 1.6 ml of stock solution were taken in a series of separate 100 ml volumetric flask and the volume was adjusted by 0.1N HCl. Absorbance was taken at 287 nm against blank for each solution.

The above 5-point concentrations were constructed to regenerate calibration curve and the results showed to achieve accuracy, reproducibility, and linearity. The relative standard deviation percent at lower concentration was 0.153. The correlation coefficient was 0.998.

Determination of suspension potency

Assay preparation: take by volumetric pipette an amount metronidazole benzoate suspension equivalent to 0.1g of metronidazole benzoate and transferred to a 100 ml volumetric flask. 50- 60 ml of 0.1N HCl was added and shaken for 45 min. The volume was made up to the mark with the same solvent and filtered with Whatman filter. 1 ml of the filtered solution was diluted to 100 ml with 0.1 N HCl solution and consequently, treatment the reference to obtain the same concentration by the same solvent.

Calculation: the absorbance of both standard and samples were measured in UV - Vis spectrophotometer that previous mentioned at 278 nm using 0.1 N HCl solution as blank. Each sample was run in duplicate and the average of absorbance reading was taken into consideration.

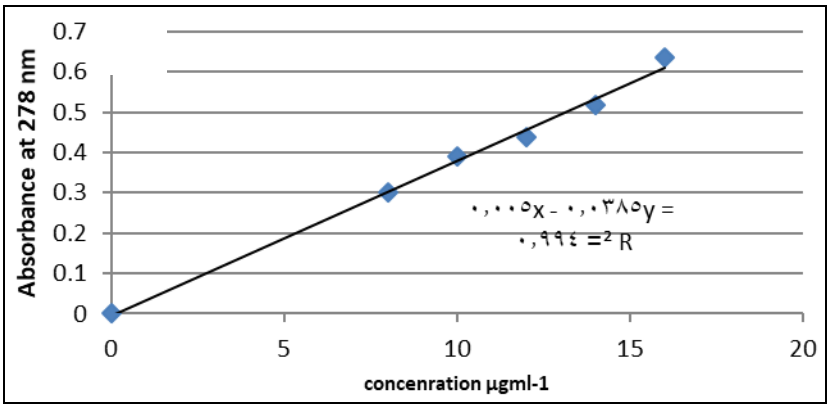

Fig. 3 Calibration curve for standard metronidazole

3. RESULTS AND DISCUSSION

This study included nine samples of metronidazole suspensions (200 mg/5 ml) that were purchased from retail pharmacies in the Western and south of the ROY (Table 1). They were evaluated in the essential parameters such as pH values, active ingredients, physical properties such as odor, color, taste by using calibrated instruments and validated method of analysis and measuring the pH values by pH apparatus.

The brands were coded as follows: MBS 01 to MBS 09. The original of these samples as follows: the samples that were coded as MBS 02, MBS 04, MBS 07 and MBS 08 are locally produced. The samples that were coded as MBS 01, MBS 05, and MBS 06 were of Arabic origin. The sample that was coded as MBS 03 was of Indian origin. And finally, the sample that was MBS 09 was reference (flagyl). The method of analysis was valid (see methodology). The suspensions products were examined according to British Pharmacopoeia and United States Pharmacopoeia [19, 20].

The Physical essential tests

The physical essential tests were carried out for suspensions brands such as redisperse, leakage, and pH tests according to BP 2008, and USP 24 (Table 2) as explained in the following:

Redisperse:

All the bottles by moderate shaking showed homogeneous distribution of the content and still enough time to make the patient takes a uniform dose. The container does not appear to adherence mass in the container either in lower of the bottle or by edge. **The leakage test** proved that all the containers sealed to accepted degree, and there are not any spot leakage and a smell of the medicines on the containers.

pH value as essential parameter

The pH value is considered essential parameter hence improves solubility and keeps drug stability at optimized conditions during shelf lives and consequently, the limit of pH value ranged between 5 to 6.5 [5]. Table 2 illustrated that the lower pH value was 4.61, and the high values were 6.05 for the brands that were coded MBS 01 and MS 09 respectively. BP and WHO determine that the pH ranges between 5 and 6.5 and consequently, all the brands gave accepted values except brand MBS 01 that gave value below the accepted limit and failed to meet criteria whereas eight brands passed this test. Our finding is comparable with the study that was conducted by Frimpong et al, (2018), in Ghana, African countries, which was entitled quality assessment of 68 brands of children's medicines such as amoxicillin and metronidazole suspensions…etc. The conclusion of this study represented that 19 samples (27.9%) failed in this paramount parameter.

From the view of pharmaceutical sciences, the pH is an essential parameter where it improves solubility and keeps drug stability at optimized conditions.

Content Uniformity

Table (2) shows the chemical assays of suspension brands products that were symbolized by MBS 01, MBS 02, MBS 04, MBS 06, MBS 08, and MBS 09 were comparable with BP specifications. Whereas brands that were coded MBS3, MBS5, MBS7 failed to meet chemical assay tests Again, MBS 1 passed in chemical assay but it failed to meet criteria in pH value as previously mentioned, and hence this increases the failure brands to four. Moreover, Table (2) also illustrates the brands that failed in quantitative analysis were in the following orders 93.81%, 86.30%, and 91.5% respectively. It could be concluded that these brands intended substandard products from the manufacturing. Again, Table (2) also shows that two brands that were coded MBS 02 and MBS 08 gave values lower to accepted limit

(96.04 and 96.33) at the time of analysis, while the expiry date according to the label bearing to the packaging is eleven and twelve months respectively. This indicates that the medicines may expire before the expiring date that is declared on the label due to both of these values were much closed to the lower accepted limit and hence, they were considered practically expired.

Moreover, these findings gave us worse expectation upon quality of children's medicine in the Yemeni market where the brands that failed were six out of eight generic brands and they constitute 75% from the study samples. Our findings were comparable with the study that was conducted by Frimpong et al (2018) that was mentioned previously. The conclusion of this study is that forty-two brands failed in uniformity contents (61.8%). The final estimated of our study proved that 75% of the samples were substandard, counterfeit and adulterated.

4. The Conclusion of these Studies

There are comparable between our findings and that conducted by Frimpong et al (2018) and Adedibu et al[22] 'as mentioned previously', ensured that adulterated, fake, and substandard medicines are available in the Yemeni market that constitute 70 to 80 %. In addition, these results obviously reflect that poor quality in children's medicines and adults. Furthermore, the CGMP and post licensed surveillance are absolutely absent in our country. Thus, the investigator advises the authority of health to build large quality control laboratories in the capital and territories for research and development to face challenges jeopardize of drug quality and apply restricted regulations that consonance with the world wide laws.

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Table 2 The results of physicochemical assays of metronidazole suspensions

Sample code	Essential Tests				
	Redisperse	pH (5 – 6.5)	Leakage	Chemical Assays (95 – 105%	Results
MBS 01	Normal	**4.61**		99.95%	Not conform
MBS 02	Normal	5.5	Comply	96.04%	Conform
MBS 03	Normal	6.01	Comply	**93.81**	Not Conform
MBS 04	Normal	5.5	Comply	102.64	Conform
MBS 05	Normal	5.5	Comply	**86.3**	Not Conform
MBS 06	Normal	5.5	Comply	102.64	Conform
MBS 07	Normal	5.5	Comply	**91.5**	Not Conform
MBS 08	Normal	6	Comply	103.5	conform
MBS 09	Normal	6.05	Comply	96.33	Conform

MBS: Metronidazole Benzoate Suspension.

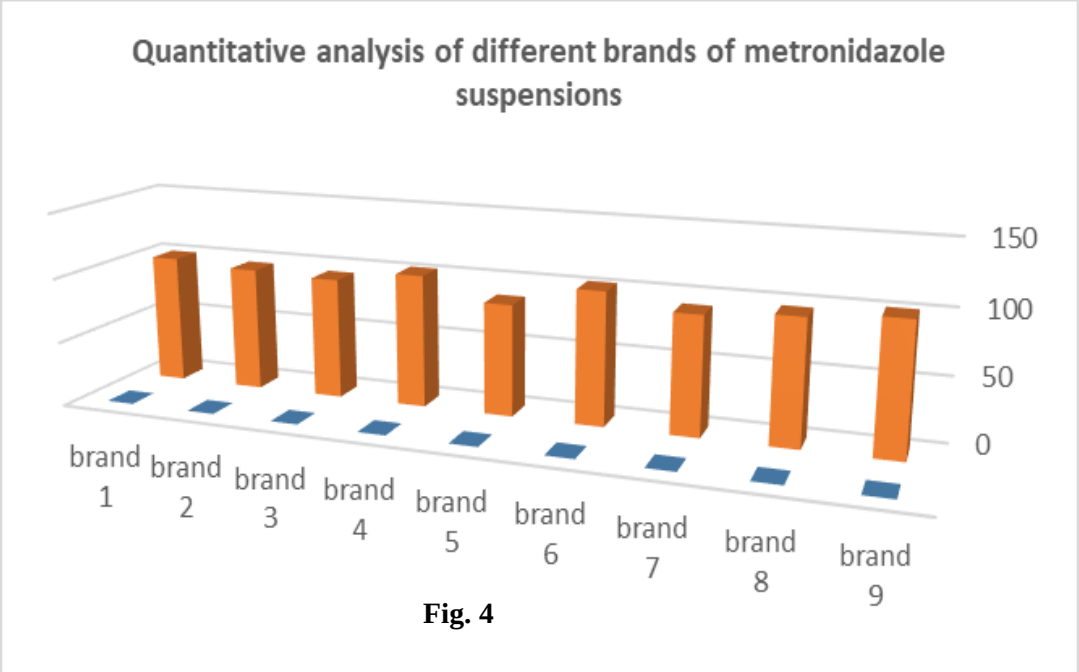

Fig. 4

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