In vitro quality assessment of ten brands of metronidazole benzoate suspensions marketed in Warri, Nigeria

Nkemakolam Nwachukwu 1* and Edwin Aboje Ubieko 2

1 Department of Pharmaceutics and Pharmaceutical Technology, University of Port Harcourt, Choba, Rivers State, Nigeria.
2 Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Madonna University, Elele, Nigeria.

Publication history: Received on 09 November 2020; revised on 17 November 2020; accepted on 19 November 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.13.2.0366

Abstract
Therapeutic failure as a result of high incidence of fake, adulterated, counterfeit and substandard drugs usage is a major concern to health practitioners, drug regulatory agencies, drug consumers and the general public in Nigeria. The objective of this study was to carry out in vitro quality assessment/evaluation of ten (10) different brands of metronidazole benzoate suspensions that are marketed in Warri, Nigeria. Metronidazole benzoate suspensions (10 brands) were purchased from some pharmacies in Warri, Nigeria. They were checked for the label information on both the secondary and primary packages, physical examination of the primary containers for tampering/breakage of seal on cap, organoleptic properties, pH, sedimentation volume, flow rate, viscosity, redispersibility and content of active ingredient/assay using standard methods. Results obtained showed that the suspensions had the necessary information on their labels, the containers were not tampered with in order to access or change their content. All the brands tested showed good results for color variation, pH, viscosity, flow rate, sedimentation, and redispersibility. All the brands met with their label claims of metronidazole benzoate content based on British Pharmacopoeia specification [95 - 105 %] except one brand (MET-A), that failed. Generally, nine of the brands representing 90 % met with their label claim and can be considered fit for distribution and consumption.

Keywords: Metronidazole benzoate; Suspension; Quality; Assessment; Pharmacies

1. Introduction
Quality assessment can be described as a process of collection of data and its analysis which enables the determination of the extent of conformity to predetermined standards. It highlights the criteria for acceptance or rejection of data. If the quality obtained through this process is not satisfactory, attempts are made to discover the reason for the shortfall in quality. Subsequently, corrective actions are introduced and the quality re-evaluated after a suitable period of time [1]. This process is dynamic and it permits the continuous revision of the set criteria and standards with the overall purpose of improving the quality of health care services.

Medicines or pharmaceutical products can be described as genuine, substandard or counterfeit [2,3]. Genuine drugs or medicines are those formulations that have met with the requirements or standards which have been stipulated for such products by the regulatory agencies while substandard drugs or medicines may be genuine products that failed to meet up with the benchmark of the quality testing protocols previously set for each product [4]. More so, such products are found to be deficient in the content of the active pharmaceutical ingredient [API]. Counterfeit drugs are described by the World Health Organization, WHO as those drugs or medicines that are deliberately and fraudulently mislabeled

* Corresponding author: Nkemakolam Nwachukwu
Department of Pharmaceutics and Pharmaceutical Technology, University of Port Harcourt, Choba, Rivers State, Nigeria.

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with respect to identity and/or source [4]. Counterfeit products include drugs with either the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging [5,6]. However, substandard drugs may not be suitably described with a blanket definition. Their definition may depend on the acceptable standards of the different countries of the world where they are either manufactured and/or used as what could be substandard in one country may be acceptable in another country. Generally, such products are bound to be ineffective, their distribution and use can result in severe untoward clinical outcomes such as no therapeutic effect and treatment failure, chances of development of resistance to antimicrobial agents especially in the treatment of bacterial infections, toxicity, undesirable side effects and even deaths [7]. Although the issue of fake, substandard or counterfeit medicines is a global phenomenon, it is mostly experienced in the third world countries [5]. Pharmaceuticals marketed in developing countries (most of which have tropical climates) have their integrity affected by poor storage conditions such as high temperature and high humidity conditions which would aid chemical degradation and alteration of the biopharmaceutical properties of the drugs that are stored before their estimated and labelled shelf lives [8]. Poverty level, chaotic drug distribution methods, unenforced drug regulatory laws has continued to favor unpatriotic charlatans whose mainstay is the profit that they would make from the sale of such products than the adverse health implications that these unwholesome drugs would have on the unsuspecting populace [9].

Pharmaceutical suspensions can be described as liquid formulations consisting of coarse dispersions of insoluble solids of a particle size range of 0.05 to 5 µm in a liquid medium [10]. Generally, they are heterogeneous and thermodynamically unstable systems made up of two phases, an internal and external phase. The internal phase usually consists of the pharmaceutical active ingredient (API) which is an insoluble powder while the external phase is mostly made up of an aqueous liquid medium. Sometimes the external phase could consist of an oily liquid. The stability of most suspensions is achieved by the addition of a single or combination of suspending agents [11] which aids in keeping the insoluble dispersed solids suspended long enough in the suspension to enable the administration of a uniform dosage whenever it is needed. Pharmaceutical suspensions can also exist as flocculated or deflocculated preparations.

Metronidazole is a nitro-imidazole derivative that has been widely synthesized in various laboratories throughout the world [12] and is quite useful as an antiprotozoal and anti-parasitic agent that is very effective in the treatment of amoebiasis, trichomoniasis, giardiasis and many other parasitic diseases [13]. It exists as yellow crystals that are slightly soluble in water, methylene chloride and alcohol [14]. Metronidazole is an essential anti-infective drug that its use cuts across every age group that is found in a community. The different classes of metronidazole that exist is dependent on the route of administration. Its popular dosage forms include: oral tablets, oral suspensions, oral capsules, topical creams, gels, and lotions and injectable. Metronidazole benzoate suspension is used mostly in the treatment of pediatric populations and occasionally in some geriatric populations especially when the patient is unable to swallow tablets.

Metronidazole is useful in the prevention of infections after an operation/surgery, treatment of infections, including infections of the blood, brain, lungs, bones, lining of the abdomen, pelvis and infections following childbirth, treatment of trichomonalis, amoebiasis, giardiasis. vaginosis, and gingivitis [15]. Its suspension and conventional release (immediate-release) oral tablets are used to treat many infections caused by bacteria or parasites. Such infections include some gastrointestinal tract (GIT) or reproductive system infections such as amebiasis and trichomoniasis respectively. Its oral extended-release tablets are useful in the treatment of vaginal infections in women [15]. Metronidazole may be used as part of a combination therapy in the treatment of some infections.

Reports of studies on in vitro quality control evaluations of metronidazole benzoate suspension and metronidazole tablets show that a certain level of unwholesome metronidazole products are in circulation in the countries where the studies were carried out [16-19]. Inadequate post marketing surveillance by both the manufacturing companies and the necessary regulatory agencies can be said to be part of the reasons for this challenge. This study was considered necessary because quality control assessments need to be carried out periodically to ensure that the metronidazole benzoate suspension at the disposal of health providers and the consumers of these products are wholesome and that the general public is not exposed to the risk of consumption of these unwholesome products which could have found its way into the drug distribution chain. Metronidazole benzoate has been one drug that is frequently prescribed for the management of gastroenteritis in children which if untreated could lead to morbidity and/or mortality within a short period of time [20].

2. Material and methods

2.1. Materials
Metronidazole benzoate (AHA Co Ltd., China), Hydrochloric acid (Sigma, USA), distilled water, Ten (10) brands of metronidazole benzoate suspension (Purchased from different pharmacies in Warri, Nigeria).

2.2. Methods

2.2.1. Collection of samples

Ten packets each of 10 different brands of metronidazole benzoate suspension were purchased from different wholesale pharmacies in Warri, Delta State, Nigeria. Selection of brands purchased was done randomly.

2.2.2. Physical evaluation of products packaging

The physical evaluation of the primary and secondary containers of the 10 different brands of metronidazole suspensions (coded MET-A to MET-J) were checked to ascertain whether they have been tampered with. This is important as the product was primarily packed in plastic screw capped bottles whose caps were designed to be tamper proof. The necessary label information such as the name and strength of the metronidazole benzoate content, National Agency for Food Administration and Control (NAFDAC) registration number, lot and batch number, the manufacturing date, expiry date, directions for use/dosage instruction, name and address of manufacturing company were also checked.

2.2.3. Organoleptic properties determination

Some organoleptic properties of the ten different brands of the metronidazole suspensions which includes the visual appearance as observed with the naked eye and odor were checked and noted.

2.2.4. pH determination

The pH of the ten different batches of metronidazole benzoate suspension was determined using a pH meter (Hannah, USA). Replicate determinations were carried out.

2.2.5. Flow rate determination

The time required for 10 ml of each brand of metronidazole benzoate suspension to flow through a graduated 10 ml glass pipette into a beaker was determined. Replicate determinations were done for each brand and the mean flow rate was calculated using equation 1:

\[ \text{Flow rate} = \frac{(\text{volume of suspension})}{(\text{flow time})} \quad \text{Eq. (1)} \]

2.2.6. Viscosity determination

The viscosity of each brand of metronidazole benzoate suspension was determined with a Brookfield viscometer (Dv2, Brookfield Engineering Laboratories, Massachusetts, USA) using Lv-02 (number 62) spindle set to rotate at 12 rotations per minute (rpm) speed, and at a temperature of 29.5 ± 0.5 °C. A 50 ml volume of each sample of metronidazole benzoate was placed in a 100 ml beaker, the spindle of the viscometer inserted into it and the instrument was switched on to establish its viscosity. This test was determined in triplicate and the average value was recorded for each sample.

2.2.7. Sedimentation volume/ratio determination

A 50 ml volume of each of the metronidazole benzoate suspensions from the ten different brands of metronidazole benzoate was collected into 50 ml graduated glass measuring cylinders after appropriate shaking of the individual product primary container. This was done in triplicates per brand and the mouth of each of the measuring cylinder containing the suspension was plugged with cotton wool and kept undisturbed on a flat shelf under ambient conditions. Observations for sedimentation volume of the particles were carried out daily on days 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28 respectively. The sedimentation ratio was calculated using equation 2 [10, 21].

\[ F = \frac{V_t}{V_o} \quad \text{Eq. (2)} \]

Where F is the sedimentation ratio, Vt is the volume of sediment formed after a given period of time while Vo is the initial volume of sediment at the time the suspensions were poured into the measuring cylinder.
2.2.8. Redispersibility determination

The redispersibility assessment was done on day 28 immediately after the last undisturbed sedimentation volume/height reading was taken and recorded. The base of the measuring cylinder was held with the left palm while the mouth was covered and held with the right palm. The cylinder was inverted in a clockwise direction through 180° [22], and then returned anticlockwise to its former position within a time of 20 ± 5 sec. This was taken as one shake. The number of shakes it took the suspensions to achieve complete redispersion of the sediment was recorded as the redispersibility number. Complete redispersion was considered to have taken place when no sediments could be seen at the bottom of the measuring cylinder [23]. The test was carried out in triplicate.

2.2.9. Standard calibration curve

A quantity of 100 mg of pure metronidazole benzoate powder was weighed using an analytical balance into a 100 ml volumetric flask. A sufficient amount of 0.1 N hydrochloric acid (HCl) was used to dissolve the metronidazole benzoate and the volume made up to the 100 ml mark of the volumetric flask with 0.1 N HCl [24,16]. Serial dilutions of the stock metronidazole solution was made to obtain solutions of 0.2, 0.4, 0.6 and 0.8 mg %. The different diluted solutions of the metronidazole benzoate and the stock solution were scanned for their absorbance readings at a wavelength of 277 nm using a model 6405 UV/Vis spectrophotometer (Jenway, UK). The absorbance readings were converted to concentrations using the Beer Lambert’s equation which is expressed as equation 3 [25]. A plot of the concentrations obtained against their absorbance readings was done.

\[ A = KC \] \hspace{1cm} \text{Eq. (3)}

Where \( A \) is absorbance, \( C \) is the concentration and \( K \) is a constant representing the slope.

2.2.10. Assay/content of metronidazole benzoate determination

Three different containers from each of the brands of the metronidazole suspension were properly shaken, uncapped and 0.5 ml of the product was measured into a 10 ml volumetric flask using a 1.0 ml pipette. It was diluted to 10 ml using 0.1N HCl. The dilutions were scanned in a UV/Vis spectrophotometer, model 6405 Jenway, UK at a wavelength of 277 nm to obtain their absorbance readings. The absorbance readings were fitted into the standard calibration plot equation to determine the concentration.

3. Results and discussion

3.1. Physical parameters

Results of the evaluation of the packaging and label information of the different metronidazole benzoate suspensions showed that the products were intact and the seal of the cap of the primary container (plastic bottle) was not tampered with. All the brands of the metronidazole suspensions had NAFDAC registration numbers or status, batch numbers, manufacturing and expiry dates, name and strength of product, locational address of manufacturer, etc. (Table 1).

Table 1 Some relevant information on the package of the metronidazole suspensions.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Manufacturing date</th>
<th>Expiry date</th>
<th>Label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET-A</td>
<td>08/2018</td>
<td>07/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-B</td>
<td>08/2018</td>
<td>07/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-C</td>
<td>09/2018</td>
<td>08/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-D</td>
<td>10/2018</td>
<td>10/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-E</td>
<td>2018</td>
<td>2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-F</td>
<td>06/2018</td>
<td>06/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-G</td>
<td>04/2018</td>
<td>04/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-H</td>
<td>09/2018</td>
<td>09/2021</td>
<td>200 mg/5 ml</td>
</tr>
<tr>
<td>MET-I</td>
<td>12/2018</td>
<td>12/2020</td>
<td>200 mg/5 ml</td>
</tr>
</tbody>
</table>
All other relevant information about each product such as the brand name, strength or concentration of metronidazole benzoate, basic information on directions for use and storage conditions were written on both the packet (secondary package), and label of the bottle (primary container). Some of the products had literature inserts containing some of the more detailed information that could not be written on the product pack or bottle label as a result of limited space. Each of the brands had a transparent 10 ml calibrated plastic dispensing cup on top of the metal cap cover of the bottle. None of the brands had any information on the type and quantity of the excipients used. All the primary containers were amber colored plastic bottles, a property that would aid to protect its content from photo degradation.

3.2. Organoleptic properties

The results of some of the organoleptic properties of the different brands of metronidazole benzoate suspension which includes the visual appearance as observed with the naked eye and odor were noted and shown in Table 2. The flavors used were mostly fruity and categorized as either orange, pineapple or raspberry flavors. This is expected as it would make the product attractive to children who consist the majority of the targeted end users. The metronidazole benzoate suspensions were mostly brightly colored, but the choice of color depended on the manufacturer.

Table 2 Organoleptic properties of metronidazole benzoate suspensions.

<table>
<thead>
<tr>
<th>Name of product</th>
<th>Appearance</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET-A</td>
<td>Lemon</td>
<td>Lemon flavor</td>
</tr>
<tr>
<td>MET-B</td>
<td>White</td>
<td>Orange flavor</td>
</tr>
<tr>
<td>MET-C</td>
<td>Pale yellow</td>
<td>Pineapple flavor</td>
</tr>
<tr>
<td>MET-D</td>
<td>White</td>
<td>Orange flavor</td>
</tr>
<tr>
<td>MET-E</td>
<td>Pale yellow</td>
<td>Lemon flavor</td>
</tr>
<tr>
<td>MET-F</td>
<td>Yellow</td>
<td>Lemon flavor</td>
</tr>
<tr>
<td>MET-G</td>
<td>Lemon</td>
<td>Banana flavor</td>
</tr>
<tr>
<td>MET-H</td>
<td>Pale yellow</td>
<td>Lemon flavor</td>
</tr>
<tr>
<td>MET-I</td>
<td>Yellow</td>
<td>Lemon flavor</td>
</tr>
<tr>
<td>MET-J</td>
<td>Orange</td>
<td>Orange flavor</td>
</tr>
</tbody>
</table>

3.3. pH

The results of the pH measurement of all the metronidazole benzoate suspensions are shown in Table 3. They were all mildly acidic and this conforms to compendia requirement which is given as 5.0 – 6.5 [14]. The pH values did not change significantly over the 28 days stability study period. This infers that the suspensions were stable and neither were they chemically or microbiologically degraded.

3.4. Flow rate

The results of the flow rates of the metronidazole suspensions are shown in Table 3. Brand MET-B suspension had the highest flow rate while MET-G did not flow out from the pipette. All the samples investigated had flow rates of < 1.0 ml/s. The flow rate is an index of viscosity which shows how pourable a suspension would be from its container as well as its ability to retain the particles in the suspension phase. The higher the flow rate, the easier it would be to remove the suspension from the container and likewise its pourability. Nine of out of the ten brands were easily pourable from their primary containers.

3.5. Viscosity

The results of the viscosity determinations are also shown in Table 3. It was observed that MET-G was the most viscous and could not be easily poured out from the product container, followed by MET-C while MET-B had the lowest viscosity. This implies that MET-G would be least flowable, least pourable and most stable of the suspensions as its
particles would remain longest in terms of suspendability before sedimentation, while the particles of MET-B would have the fastest sedimentation rate. The extent of viscosity experienced would depend on the type and amount of polymer used as suspending agent in the formulation. Suspending agents are meant to retard the rate of sedimentation of particles in the suspension as well as giving a mouth feel to the preparation. The viscosity results of the suspensions can be correlated to their flow rates (Table 3). The lower the viscosity value, the higher the flow rate that was observed in the metronidazole benzoate suspension.

Table 3 Flow rate, pH, viscosity and redispersibility of metronidazole suspensions.

<table>
<thead>
<tr>
<th>BRAND</th>
<th>Flow rate (ml/s)</th>
<th>pH</th>
<th>Viscosity (cP)</th>
<th>Redispersibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET-A</td>
<td>0.23 ± 0.01</td>
<td>6.70 ± 0.20</td>
<td>475.00 ± 0.89</td>
<td>18.30 ± 2.89</td>
</tr>
<tr>
<td>MET-B</td>
<td>0.94 ± 0.11</td>
<td>6.51 ± 0.13</td>
<td>75.53 ± 0.05</td>
<td>16.61 ± 2.08</td>
</tr>
<tr>
<td>MET-C</td>
<td>0.20 ± 0.01</td>
<td>5.52 ± 0.10</td>
<td>665.00 ± 1.15</td>
<td>22.65 ± 1.53</td>
</tr>
<tr>
<td>MET-D</td>
<td>0.49 ± 0.02</td>
<td>6.21 ± 0.11</td>
<td>125.05 ± 2.04</td>
<td>5.60 ± 0.58</td>
</tr>
<tr>
<td>MET-E</td>
<td>0.25 ± 0.00</td>
<td>5.41 ± 0.30</td>
<td>428.50 ± 3.06</td>
<td>30.30 ± 1.55</td>
</tr>
<tr>
<td>MET-F</td>
<td>0.29 ± 0.01</td>
<td>5.35 ± 0.09</td>
<td>380.00 ± 2.55</td>
<td>6.30 ± 0.58</td>
</tr>
<tr>
<td>MET-G</td>
<td>NF</td>
<td>5.70 ± 0.06</td>
<td>1228.40 ± 2.96</td>
<td>NA</td>
</tr>
<tr>
<td>MET-H</td>
<td>0.37 ± 0.02</td>
<td>5.41 ± 0.20</td>
<td>337.50 ± 2.23</td>
<td>4.00 ± 0.01</td>
</tr>
<tr>
<td>MET-I</td>
<td>0.44 ± 0.00</td>
<td>4.60 ± 0.30</td>
<td>152.50 ± 1.10</td>
<td>2.50 ± 0.35</td>
</tr>
<tr>
<td>MET-J</td>
<td>0.29 ± 0.01</td>
<td>6.00 ± 0.08</td>
<td>382.50 ± 3.13</td>
<td>20.34 ± 3.78</td>
</tr>
</tbody>
</table>

Key: NF represents no flow while NA represents not applicable.

3.6. Sedimentation volume/ratio

The results of the sedimentation volume or ratio of each of the sediments of the insoluble components that were suspended in the metronidazole benzoate suspensions over a 28 day period of observation is shown in Fig.1. At the commencement of the study, all the particles were suspended in the measuring cylinder with sedimentation ratio of one (1) but when stood undisturbed on the shelf for the duration of the study, various volumes/ratios of sediments of the particles were observed. Generally, the sedimentation ratio decreased as the days increased for all the suspensions except in MET-G where the particles continuously remained completely suspended throughout the 28 days of the study. Closely following MET-G were MET-H and MET-F which showed very little sedimentation from day 4 to day 28.

![Figure 1 Sedimentation ratio of metronidazole suspensions](image)

The sedimentation volume or ratio can be related to the viscosity of the individual suspensions [21]. Those with a high viscosity had their particles suspended in the suspensions much longer than those that were less viscous. This is because
the viscous environment caused a reduction in the sedimentation rate of the particles. Besides the viscosity, the rate of sedimentation could be related to the particle size of the insoluble solids in the suspension and the zeta potential. The larger the particle, the greater is the tendency to sediment while smaller particles could stay suspended for a longer period of time [26]. Suspension MET-D showed a gradual but steady decline from day 2 to 28. The other suspensions showed a sharp decline between days 2-5, and thereafter remained stable till day 28. Most of the suspensions were flocculated and their particles did not form a cake at the bottom of the measuring cylinder. The suspensions were considered stable based on the ability of the particles to remain suspended within the suspension long enough after agitation to permit the withdrawal of a uniform dose each time the formulation is to be administered. An oral suspension is considered stable and more acceptable when the sedimentation value approaches a value of one [27]. Generally, the steeper the regression line, the less stable is the suspension. The order of stability is MET-G>MET-H>MET-F>MET-D>MET-J>MET-C>MET-E>MET-I>MET-B>MET-A.

3.7. Redispersibility

The redispersibility numbers obtained from the redispersibility test of the different metronidazole suspensions are shown in Table 3. All the metronidazole suspensions were redispersible after 28 days on the shelf. However, their degree of redispersibility varied from one brand to another. It was observed that the metronidazole benzoate suspensions were redispersible. A good suspension is easily redispersed (low redispersibility number) so as to ensure uniformity of administered doses of medicaments upon shaking or agitation [26]. This implies that flocs of the particles were formed upon sedimentation which permitted the easy flow of the external phase into them thereby enhancing easy redispersion on agitation.

3.8. Assay/content of metronidazole

The results of the assay/content of metronidazole benzoate available in the commercial brands of the suspensions are shown in Figure 2. MET-H had the highest content of metronidazole benzoate (100.05 %) while MET-A which contained 92.61 % had the least value. All the brands except MET-A met with their label claims. Thus nine brands representing 90.00 % of the total samples evaluated complied with the assay/content of active ingredient test as stipulated by the British Pharmacopoeia (BP) while one brand representing 10.00 % of the samples assayed failed the test. According to the BP, suspensions containing metronidazole benzoate should contain not less than 95.00 % or more than 105.00 % of metronidazole benzoate [14].

Figure 2 Assay/Content of metronidazole benzoate in the suspensions.

4. Conclusion

The physical evaluation of the containers of the metronidazole suspensions showed that the integrity of the seal of the caps of the bottles which served as the primary container were not tampered with which affirms the integrity of the product as was made by the manufacturer. All the ten brands of the suspensions had the relevant information concerning the product on their labels. All the drugs were registered by NAFDAC and were manufactured in Nigeria. Evaluation results of the metronidazole benzoate suspensions based on the standard assessment criteria such as pH showed a consistent data throughout the 28 days period implying that the metronidazole benzoate suspensions were...
The suspensions were mildly acidic with values similar to the pharmacopoeia pH value of metronidazole (BP, 2012) which is suggestive that the formulation excipients did not adversely interact with the metronidazole and neither was any of the products microbiologically degraded. The flow rate and viscosity also supported the stability of the products. Nine (90 %) of the metronidazole benzoate suspensions were pourable from the container as well as viscous enough to cause the suspended insoluble particles of the formulation to remain suspended long enough that would permit adequate and consistent withdrawal of these particles as a uniform dose after shaking and re-dispersing the sediments of the suspension as well as giving the consumer a good mouth feel. The sedimentation volume or ratio were generally < 1, and the particles settled in a manner that permitted the easy flow of the vehicle within the floccules which enabled easy loosening and total redispersibility within a short period of time. The content of metronidazole obtained after assay showed that 90 % of the products contained their label claims of metronidazole benzoate while other physical parameters which were evidences of a stable and pourable product show the good quality or standard of these commercial products. Thus, it can be seen from the results obtained that most of commercial brands of metronidazole benzoate being marketed in pharmacies in Warri are of good quality and capable of eliciting the expected therapeutic response that is expected of them when correctly administered.

Compliance with ethical standards

Acknowledgments

The authors are grateful to Mr. Sampson Bekweri of the department of Pharmaceutics and Pharmaceutical Technology, University of Port Harcourt for technical assistance during this work.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

References


