Open Access Research Journal of
Chemistry and Pharmacy
Journals home page: https://oarjpublication/journals/oarjcp/
ISSN: 2783-0276 (Online)

OARJ OPEN ACCESS RESEARCH JOURNALS

(RESEARCH ARTICLE)

Analysis of injectable drugs (Pentazocine and Diazepam) sampled from shops and companies in Anambra State, Nigeria

John A. Ezugwu [1,*], Sylvia I. Okonkwo [1], Edith C. Ezugwu [2], Okonkwo C. Kenechukwu [3] and Chinwe C. Okonkwo [4]

[1] *Department of Pure and Industrial Chemistry, Chukwuemeka Odumegwu Ojukwu University Anambra state Nigeria.*
[2] *Department of Biochemistry Nnamdi Azikiwe University Awka Anambra State Nigeria.*
[3] *Department of Medicine and Surgery Kaplan Medical Test and Prep Institute USA.*
[4] *Department of Medicine and Surgery Caribbean Medical University, Curaçao USA.*

Open Access Research Journal of Chemistry and Pharmacy, 2023, 03(02), 026–048

Publication history: Received on 09 April 2023; revised on 01 June 2023; accepted on 04 June 2023

Article DOI: https://doi.org/10.53022/oarjcp.2023.3.2.0058

Abstract

The incidences of drug adulteration and drug resistance have been a major problem facing mankind in recent years. Low and medium-income countries like Nigeria are most affected. An investigation was carried out to determine the quality of Pentazocine and Diazepam injections in circulation in Anambra State. The injections were sampled from different markets, shops, and pharmaceutical companies within the State. Imported and locally manufactured brands of the injections were analysed. Identification, pH, Assays, Bacteria Endotoxin, sterility, and sub-visible particulate matter contamination were analysed. The results of the Identification, Bacteria Endotoxin (BET), Particulate matter count, and pH are all within the British Pharmacopeia. The Pentazocine Assays obtained were (98.31%- 102.5%) which are within the British Pharmacopeia limit of (95% – 105%). This is good for health management. The Assays of two of the Diazepam injections tested were between (111.33% - 111.53%), which is above the approved British Pharmacopeia limit of (90%-110%). This is an indication of high level of impurities and not good for health care management.

Keywords: Pentazocine; Diazepam; Adulterated Drugs; Sterility Test

1. Introduction

A medicine or device is said to be adulterated, if, the methods, facilities, or controls used for its manufacturing, processing, packaging or, storing do not conform to the accepted standard current good manufacturing practice (cGMP) to assure that such drug meets the specified requirements as it concerns safety, identity, strength, quality, purity, and characteristics, which it purports or represented to possess.[1] The burden of fake and adulterated drugs in circulation in Anambra State is a big issue in the economy of the state.[2] Anambra state houses the largest drug market in West African sub region and as such has the highest burden of the fake and falsified injectable drugs in Nigeria.[3,4] . Pentazocine is a non-narcotic analgesic and it belongs to the class of medicines known as opioids. It is used for the management of moderate to severe pains. It can also act as a weak antagonist. [5].

Pentazocine can be addictive and can be abused as a result of its addictiveness. Pentazocine abuses in Nigeria are increasing and no concrete intervention has been put in place to curb the menace. [6].

Pentazocine is very effective and when used with appropriate caution and monitoring are also generally safe. They not only relieve sensation of pain but also relieve the effective and physiologic reaction to pain and thus reduce patient anxiety as a result, it is most of the times abused [7]. Pentazocine hydrochloride is applicable for oral use and the lactate form is made use of for parenteral and rectal medication.[8].

* Corresponding author: John A. Ezugwu

Copyright © 2023 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

In Nigeria, despite regulation, pentazocine is still being purchased "over the counter" in many places. Fake and adulterated Pentazocine is rampant in the Nigeria drug market because of its high demand by medical practitioners for different medical purposes.[9].

Diazepam is known with the commercial name of Valium. It is a benzodiazepine derivative. The chemical name of diazepam is 7-chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin2-one. It is a colorless to light yellow crystalline compound, insoluble in water. The empirical formula is $C_{16}H_{13}ClN_2O$ and the molecular weight is 284.75.[10].The compound is recognized for its tranquilizing, entrancing, anxiolytic, muscle relaxant, and anticonvulsant possessions. Diazepam acts fast and very effective anxiolytic prevalent in application owing to the medicines extensive healing index, low harmfulness, and enhanced protection profile. [11]. Diazepam is popular and regularly used medicine in Nigeria because of its numerous applications in handling several medical conditions; as a result, some of the brands in the Nigeria medicine market adulterated and falsified and Anambra State in particular has a high burden of the falsified medicines.[12]. Nation Agency for Food and Drug Administration and Control (NAFDAC) is the body responsible for the regulation of both locally manufactured and imported medicines in Nigeria. The agency is doing its best to checkmate the ingress and circulation of medicines and food items in the country, but the effort has not yielded much result in stopping the ugly incidence.

Thus, the importance of conducting this study of analysis of injectable drugs in circulation in a population dense state is very important. The present study was conducted at a parenteral drug manufacturing company, Juhel Nigeria Limited in Awka Anambra State capital. In the study, Identity test, pH, Sterility, Bacteria Endotoxin Test (BET), particulate matter contamination, and Assays were analysed in 900 ampoules of sixty brands of Pentazocine injection and 900 ampoules of sixty brands of Dizepam Injection. The results obtained from these analyses were compared with British Pharmacopeia limits for the two injections. This serves as a basis for further studies in the area of the quality of injectable medicines in circulation in the State and in the nation at large.

2. Materials and methods

2.1. Study Area

The study areas were Awka, Onitsha and Nnewi (6.2220° N, 7.0821° E, 6.1329° N, 6.7924° E,6.0105° N, 6.9103° E) in Anambra State, Nigeria where the sampling were at Eke Awka market and Pharmacy shops within Awka town, Onitsha Head bridge drug Market and pharmacy shops within Onitsha town, and Nkwo Nnewi drug market and pharmacy shops within Nnewi town.

2.2. Drug Sampling

Samples of one sixty (60) brands of Pentazocine injection and sixty (60) brands of Diazepam injection were purchased from notable pharmaceutical stores and open drug markets in the above cities in Anambra State.

The drugs were purchased in the three senatorial districts of Anambra State which are Awka town in Awka South Local Government Area. These were purchased at Eke Awka Medicine Market and other Pharmacy Shops in and around Awka. The second sets of drugs were purchased at the Nnewi drug market and other pharmacy shops in and around Nnewi. The third sets of medicines were purchased at Onitsha Head Bridge Drug market and other pharmacy shops in Onitsha.

The medicines were taken to Juhel Nigeria Limited Laboratory and kept at the temperature of 25°C, protected from light.

- Fourier Transformer Infrared (FTRI) Spectrometer (Bruker Vector 33, Model No: I18500 PS15)
- High-Performance Liquid Chromatography (Agilent Technology, Model No: 1200)
- Ultraviolet-Visible Spectrophotometer (Pharmacia Biotech Model No: 80-2106-20)
- Laminar Air Flow (LAF) (AirPac)
- Vortex mixer (Shanghai Leeuwen Scientific Instrument, Model No: XH-B)
- Hot air Oven (Thermo lab Equipment, Model No: TO 00003256)
- Autoclave (Shivani Scientific Equipment, Model No: 19400)
- Absolute Ethanol. (JHD)
- Isopropyl Alcohol
- Ethyl acetate (SDFCL)
- Ammonia (Griffin & George)

- Acetonitrile (Merck)
- Refrigerator (Samsung, Model No: 27784DAS200167N)
- Biological Oxygen Demand (BOD) Incubator (Thermolab Equip, Model No: TB 000080G)
- Bacteriological Incubator (Thermolab Equipment, Model No: TI00400G)
- Hot Air Oven (Thermolab Equipment, Model No: TO 00003256)
- pH meter (Model No: PHS-3C 600410089007)

2.3. Preparation of samples and analysis

2.3.1. Preparation of Sample for Assay Test

All the samples for assay analysis were washed exteriorly with 70% Isopropyl alcohol (IPA)and rinsed with distilled water before setting them up in the appropriate places for analysis. The standards and the samples for the HPLC and UV-Vis spectroscopic analysis for assay were prepared using British Pharmacopeia Certified reference standards (BPCRS) and laboratory working standards (WS).

2.3.2. Preparation of samples for Sterility Test

The exterior surfaces of containers of the samples were cleaned with 0.1 % liquid soap and distilled water. A stainless trolley used for carrying the samples was sanitized with 70%IPAsolution. The samples were transferred into a buffer room; the sample containers were dipped in 70% IPA solution for 15–30 minutes. All equipment were wiped with 70% IPA solution to make them sterile. The Laminar Air Flow bench was washed down thoroughly with 70% IPA before and after testing. All the Media that were used were tested for growth promotion.

2.3.3. Sample Preparation for Bacteria endotoxin Test

The Maximum Valid Dilution of the samples that were tested were checked and the samples were diluted accordingly. The appropriate numbers of vials of the samples tested were opened. The samples were pooled into an appropriate sterile pyrogen-free container and an empty sterile pyrogen-free appropriate sized container was taken and labeled with the dilution factor used in the test and batch number of the product. The required volume of the sterile pyrogen-free water was added into the container and then added to the appropriate amount of the pooled sample to prepare the desired dilution. The diluted product sample was vortexed for at least 30 seconds to mix it thoroughly, and then 2ml of the diluted sample was taken in a clean test tube and the pH of the sample checked. The pH of the samples were in the range of 6.0 to 7.5. Excessive dilution of the sample was avoided. Only 10% or less dilution was done. The dilution is carried out because the Bacteria Endotoxin limit of the sample is higher than the sensitivity of the Lysate [13].

2.3.4. Sample Dilution Steps for Bacteria Endo Toxin (BET)

Dilution Step for Pentazocine Injection (8.7EU/ML)

- 1000µl of sample + 740µl of (LRW) = 5.0EU/ML
- 100µl of sample + 1900µl of LRW = 0.25EU/ML Test solution
- 1000µl of step 1 + 1000µl of LRW = 0.125EU/ML Test solution

Dilution Step for Diazepam Injection (58EU/ML)

- 100µl of sample + 1060µl of LRW = 5.0EU/ML
- 100µl of sample + 1900µl of LRW = 0.25EU/ML Test solution
- 1000µl of step 1 + 1000µl of LRW = 0.125EU/ML Test solution

Note: LAL =Limulus Amoebacyte Lysate

EU/ML = Endotoxin Unit/ml

LRW = LAL Reagent Water

2.3.5. Sample Preparation for Particulate Matter Contamination

The sample ampoules were thoroughly washed with a 1% soap solution. After that, they were rinsed with filtered 70% isopropyl alcohol to remove any particles on the ampoules. This was repeated three times and the ampoules were

allowed to dry. Acetone was taken into the sampling bottle to clean the bottle of any particle or contamination. The syringe and sampler were cleaned by rinsing with Isopropyl alcohol using the syringe clean button.

2.4. Assay Determination

2.4.1. Pentazocine Injection Assay

The assay determination for Pentazocine is by UV-Vis spectrophotometry method

Ultraviolet-Visible spectrophotometer (UV-Vis Spec.) (Pharmacia Biotech Model 80-2106-20) was used for the analysis of Pentazocine Assay.

- Standard Solution

150 mg of Pentazocine working standard was accurately weighed into a 100 ml volumetric flask; 50 ml of the blank was added and shaken well. The solution was sonicated for 5minutes and made up with the same solvent then, shaken well. The solution was sonicated for another 5minutes. From the resulting solution, 5ml was taken into 100ml volumetric flask; 50 ml of blank was added to 100ml with blank.

$$150\ mg\ \rightarrow 100\ ml\ \xrightarrow{5\ ml} \rightarrow 100\ ml \ldots\ldots\ldots\ldots (3.1)$$

- Sample Solution

10ml of the sample was taken into a 100 ml volumetric flask,50 ml of the blank was added and shaken well. The solution was sonicated for 5mins and made up with the same solvent and then shaken again. The solution was sonicated for another 5mins. From the resulting solution, 5ml was taken into a 100ml volumetric flask and diluted to 100ml with blank.

$$10\ ml\ \rightarrow 100\ ml\ \xrightarrow{5\ ml} \rightarrow 100\ ml \ldots\ldots (3.2)$$

Correction Factor: 0.121

Blank: 0.1M HCl

- Assay Calculation

Wavelength at 279 nm

Weight of the standard **(W)** (_) g

Potency of the standard **(P) (**_) %

Absorbance of the sample solution **(A)**(a__, b__,c__).Average (A) (_)

Absorbance of the standard solution **(B)**(a__, b__,c__).Average (B) (_)

$$Estimated\ amount\ (E): = \frac{(A)-0.121}{(B)} \times \frac{(W)}{10} \times (P) = (\_)\%w/v \qquad (3.3)$$

$$\%\ Label\ Claim\ Assay: = \frac{(E)}{1.5} \times 100 = (\_)\%, \ldots\ldots\ldots\ldots (3.4)$$

The absorbance was measured at 279 nm.

2.4.2. Diazepam Injection Assay

HPLC (Agilent model LC 1200 series) was used for the analysis of Diazepam Injection Assay

Chromatographic condition:

Column: 250mm × 4.60cm, 5µ, C_{18} column

Flow rate: 1.5ml per minute

Detection Wavelength: 230 nm

Injection volume: 2.0µl

Mobile Phase: A mixture of 400 ml of Methanol: 200ml of Acetonitrile and 400 ml of Potassium dihydrogen phosphate 0.05M (adjusted the 0.05M KH_2PO_4 to pH 3.0 with orthophosphoric acid before mixing up with other solvents).

- Standard Solution

25mg of Diazepam British Certified Reference Standard (BCRS) was weighed into a 100 ml volumetric flask and dissolved with the mobile phase; the solution was sonicated and made up to 100 ml with the mobile phase.

25mg ⟶ 100ml

- Sample solution

5 ml of the sample was accurately pipetted into a 100 ml volumetric flask and diluted to 100 ml with the mobile phase and sonicated for 5 minutes.

5ml ⟶ 100ml

$$Estimated\ amount\ (C) = \frac{\text{Peak area of the sample solution x (Wgt of standard) x (potency)}}{\text{Peak area of the standard solution x 5}} \quad \text{....(3.15)}$$

- Assay Calculation

Detection wavelength 254 nm

Weight of the standard **(W)** ( )g

Potency of the standard **(P)** ( )%

Peak area of the sample solution (A)(a__, b__, c__).Average (A) (_)

Peak area of the standard solution (B)(a__, b__, c__).Average (B) (_)

Volume in ml of the sample solution (V): ( )

Estimated amount **(E)** =

$$\frac{(\text{A})\ x\ (W)\ x\ (P)}{(\text{B}) \text{x } 4 \text{ x } 100} = (\_)\%\ w/v \quad \text{.....(3.16)}$$

$$\%\ Assay = \frac{(\text{E})}{0.5}\ x\ 100 = (\_)\% \text{.....(3.17)}$$

2.5. Sterility Determination

Approximate contents of the sample vials were aseptically transferred into the sterility testing funnel of the testing unit. The pooled samples were then drawn through a membrane with the help of a vacuum pump and washed with an appropriate number of rinses of diluents. The membrane filters were aseptically cut off into two equal parts, one part was placed into 100 ml sterile Fluid Thioglycollate Medium and the other part into 100 ml sterile Soya Bean Casein Digest Medium. Two tubes, each from the different fluid media were used for negative control by handling as in sterility testing tubes but without the addition of membrane. Fluid Thioglycollate media tubes were incubated at 32.5°C for a minimum of fourteen (14) days. Soya Bean Casein Digest media tubes were incubated at 22.5^{0}C for a minimum of fourteen (14) days.

2.6. Interpretation of Sterility Test Result

Daily during and after the incubation period, all incubated tubes were examined for macroscopic evidence of microbial growth, such as turbidity and surface growth. If no evidence of microbial growth was observed, the samples tested complied with the test for sterility. If evidence of microbial growth was found, the sample examined does not comply with the test for sterility, unless it can be demonstrated that the test was invalid for causes unrelated to the sample examined. The Pentazocine injection brands tested, five (5) out of the sixty brands showed growth in the media. Eight

(8) brands of the Diazepam Injections had growths in them. These results for Pentazocine and Diazepam followed a more or less similar trend with the study conducted by Udegbunam *et al*, [14] in Nsukka Enugu State Nigeria.

2.7. Bacteria Endotoxin Determination

Endotoxins are the most common cause of toxic reactions resulting from contamination of pharmaceutical products with pyrogens; their pyrogenic activity is much higher than most pyrogenic substances. These endotoxins are lipopolysaccharides originating from the cell wall of gram-negative bacteria. The conclusion is generally justified that the absence of bacterial endotoxins in a product implies the absence of pyrogenic components, provided the presence of non-endotoxin pyrogenic substances can be ruled out. However, the presence of endotoxins in a product may be masked by factors interfering with the reaction between the endotoxins and amebocyte lysate. A pyrogen-free product is a material that exhibits a lower endotoxin concentration as determined by the FDA or a pharmacopoeia. The content of bacterial endotoxins is expressed in EU / g (endotoxin unit) or in IU / g (international unit), the conversion relationship between the two units being: 1 IU / g = 1 EU / g. Since the Endotoxins are serious threat to pharmaceutical formulations, it is therefore necessary to validate Bacteria Endotoxin of each of the injections and be able to quantify the extent of endotoxin per ampoule of the injection. Two methods were applied in determining the endotoxin content in the injections.

2.7.1. Gel Clot Method.

All the analysis was performed under a Laminar Air Flow to keep the environment free from contamination. 100 µl of the samples analyzed were pipetted into de-pyrogenated test tubes in duplicates. The negative control was prepared by adding 100 µl of LAL Reagent Water to a de-pyrogenated test tube. The positive control was prepared by adding 50 µl of the sample being analysed and 50 µl of control Standard Endotoxin to a de-pyrogenated test tube. 100 µl of LAL reagent water was added to each test tube. All tubes were incubated at 37^0C for 60 minutes. After incubation, all tubes were visually inspected for the presence of a firm gel clot by inverting the tubes.

2.7.2. Kinetic Chromogenic Technique

- One ampoule of the endotoxin standard containing 5.0EU/ml of endotoxin stock solution was reconstituted with 1060µl of LAL reagent water as indicated in the certificate of analysis to make a solution of 50EU/ml stock solution and vortexed for 15 minutes. The resulting endotoxin was diluted serially to 0.005EU/ml by taking 0.1 ml containing 50Eu/ml at each step for four times and adding to 0.9ml of LAL reagent water.
- 100µl of the LAL reagent water was carefully dispensed into endotoxins standards and the injection samples were also dispensed into the appropriate wells of microplates.
- Endotoxins were added to the wells designated on the templates for product positive control (PPC) in the following order: 10µl each of the 50EU/ml, 5EU/ml, and 0.5EU/ml endotoxins standards were added into each of the PPC wells containing 100µl of the product sample as directed by the assay template.
- Each well of the product sample also contained 100µl of 5EU/ml, 0.5EU/ml, or 0.05EU/ml solutions respectively.
- Filled plates were placed in the microplate reader and closed with the lid. The plates were Pre-incubated in dry block incubator for 10mins at 37˚C ±1˚C.

Near the end of the pre-incubation period, more LAL reagent vials were reconstituted with sufficient LAL reagent water as specified by the manufacturer and mixed gently and thoroughly without being vortexed. The reagent was pooled into a reagent reservoir and mixed gently rocking from side to side. The microplate reader was opened using an 8-channel multi pipette, and 100µl of the LAL reagent was dispensed as quickly as possible, avoiding bubbles strictly into all the filled wells and the microplate, beginning with the first column (A1-111) and proceeding in sequence to the last column used.

- The micro plate cover was removed while the assay was performed and the OK button on the computer keyboard was clicked on to initiate the test.
- Result reading: The results were displayed on the computer monitor indicating whether each of the products was within the endotoxin release limit by showing the quantity of the endotoxin contained in each of the injection, and subsequently printed out.

2.8. Determination of Sub-visible particulate matter contamination

Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas, bubbles, unintentionally present in the solutions. These particulate matters were determined thus; the samples were placed at the sampling point of the liquid particle counter and the syringe of the sampler was lowered each time for sampling and analysis. The counter uses laser light for light obstruction count. As the liquid passes through the laser

any obstruction along the light beam is counted as a particle. These procedures were repeated three times on each sample. The average cumulative count of the three samples was taken, the results were printed and calculation of the particulate matter was made for each of the samples. After analysis, the syringe was lowered to unload the syringe.

2.9. Determination of leakages

20 Ampoules each of the samples were taken and kept in a basin, and1000 ml of 0.1% methylene blue solution was poured in the basin and placed in a vacuum oven, the vacuum oven door was closed to dip the ampoules and a vacuum of 700 mm was applied and maintained for 10 minutes. The vacuum was released and the ampoules were cleaned and checked for any blue colour percolation into the ampoules. The observation was recorded

2.10. Statistical analysis

Microsoft Excel was used to determine statistical data and the results were all presented as the mean (±) standard deviation.

3. Results and discussion

3.1. Identification Determination

Identification testing establishes the identity of the drug substance(s) in the drug product and be able to discriminate between compounds of closely related structure which are likely to be present[13] (European Medicines Agency 2000). Identification tests were carried out on both the Pentazocine and Diazepam injections to ascertain the true identities of the injections. All the Pentazocine injection identification was done by thin Layer Chromatography. Silica Gel F_{254} recoated plate was used as the stationary phase. A mixture of 10 volumes of 18 M ammonia, 10 volumes of ethyl acetate 30 volume of water, and 50 volumes of absolute ethanol was used as the mobile phase. The solvent front was allowed to ascend 10 cm above the line of application. 5 µl each of the injections containing an equivalent of 0.75%w/v of Pentazocine and 0.4 %w/v of calcium lactate in methanol solutions were applied. After removal of the plate, it was allowed to dry in the air, and it was sprayed with a 1% w/v solution of potassium permanganate and examined in daylight. The principal spot in the chromatogram obtained with the 0.75% Pentazocine injection of all the brands were similar in colour, position, and size to that in the chromatogram obtained with the 0.4%w/v of the calcium lactate in ethanol solution.

2 ml of Diazepam injection was taken from each of the brand into a separator, and 20 ml of water was added shaken, and 20 ml of chloroform was added, and shaken vigorously for 2 Minutes. The chloroform layer was filtered through about 5 g of the anhydrous granular sodium sulfate into a beaker. The chloroform extract was evaporated on a steam bath with the aid of a current of air to dryness. The residue was dissolved in 20 ml of anhydrous ether, filtered, and evaporated the filtrate to dryness using a current of air. The oily film was vigorously scraped with a spatula, and dried in a vacuum over phosphorus pentoxide at 60 °C for 4 hours: the IR absorption spectrum of a potassium bromide dispersion of the residue exhibits maxima only at the same wavelengths as that of a similar preparation of Diazepam working Standard for all the brands of the Diazepam injection.

3.2. Hydrogen potential (pH) Determination

The injections were tested for hydrogen Potential (pH). The pH has an effect on the permeation of drugs in the body [14] [15] range of 1 pH unit equals a 10-fold change in the concentration of hydrogen ions. The pH of human blood is about 7.35. Any changes in pH (even those that seem insignificant), effect great changes in the hydrogen ion concentration [16].The need to determine the pH of the brands of the injections in circulation within the State. The pH for Pentazocine and Diazepam are not strongly acidic within, 4.0 – 5.0, and 6.2 -6.9, respectively for optimal performance within the human system. The results of the pH for the two injections are as shown in the table 1 and 2 below. Four of the Pentazocine injection failed the pH test; the results were 5.10, 3.87, 3.91 and 3.93 against the pharmacopeia limit as stated above. Six of the Diazepam injections were not within the pharmacopeia specifications. The results of the failed injections are 6.0,6.18, 5.08, 7.66, 6.11, and 6.0. The implication of this failure is that these injections will affect the hydrogen ion concentration of the body when used on patients. The result followed a These results followed a similar trend with the study conducted by ogah *et al*, [17] in Lafia Nasarawa State Nigeria.

Table 1 Results of the pH of Pentazocine Injection and Diazepam Injection

S/N	Pentazocine Injection Brands	BP Specification of pH of Pentazocine Injections (4.0 – 5.0)	Diazepam Injection Brands	BP Specification of pH of Diazepam Injections (6.2 -6.9)
1	1a	4.68±0.001	1a	6.58±0.01
2	2a	4.61±0.001	2a	6.67±0.01
3	3a	4.59±0.001	3a	6.30±0.01
4	4a	4.75±0.001	4a	6.91±0.01
5	5a	4.7±0.001	5a	6.67±0.01
6	6a	4.53±0.001	6a	6.00±0.00
7	7a	4.71±0.001	7a	6.60±1.08
8	8a	4.07±0.006	8a	6.67±1.08
9	9a	4.01±0.006	9a	6.18±0.00
10	10a	4.23±0.006	10a	4.43±0.00
11	11a	4.14±0.012	11a	5.08±0.00
12	12a	4.00±0.00	12a	6.30±0.00
13	13a	4.08±0.00	13a	6.27±0.00
14	14a	4.51±0.006	14a	6.39±0.00
15	15a	4.80±000	15a	6.37±0.00
16	16a	4.78±0.012	16a	6.45±0.00
17	17a	4.40±0.006	17a	6.26±1.08
18	18a	4.31±0.00	18a	6.19±0.00
19	19a	5.10±0.00	19a	6.37±0.00
20	20a	3.87±0. 006	20a	6.41±0.00
21	1b	4.51±0.012	1b	6.38±0.00
22	2b	4.56±0.006	2b	6.28±0.00
23	3b	4.60±0.011	3b	6.50±0.00
24	4b	4.78±0.005	4b	6.51±1.08
25	5b	4.88±0.012	5b	6.66±0.00
26	6b	4.12±0.00	6b	6.74±1.08
27	7b	4.90±0.00	7b	6.57±0.00
28	8b	4.59±0.00	8b	6.53±0.00
29	9b	4.43±0.00	9b	7.66±0.00
30	10b	4.54±0. 006	10b	6.39±0.00
31	11b	4.77±006	11b	6.31±0.00
32	12b	3.91±006	12b	6.54±0.00
33	13b	4.03±0.00	13b	6.60±1.08
34	14b	4.90±006	14b	5.90±1.08

35	15b	4.65±0.00	15b	6.88±0.00
36	16b	4.21±006	16b	6.63±0.00
37	17b	5.00±006	17b	6.41±0.00
38	18b	4.44±006	18b	6.65±1.08
9	19b	4.60±006	19b	6.61±0.00
40	20b	4.94±006	20b	6.79±0.00
41	1c	4.10±006	1c	6.50±0.00
42	2c	4.44±0.00	2c	6.49±1.08
43	3c	4.47±0.00	3c	6.11±0.00
44	4c	4.80±0.00	4c	6.40±1.08
45	5c	4.14±0.00	5c	6.60±1.08
46	6c	4.00±0.006	6c	6.80±0.00
47	7c	4.53±0.01	7c	6.44±0.00
48	8c	4.21±0.006	8c	6.35±1.08
49	9c	4.03±0.01	9c	6.77±0.00
50	10c	4.96±0.04	10c	6.00±0.00
51	11c	4.79±0.01	11c	6.90±1.08
52	12c	3.93±.00	12c	6.61±0.00
53	13c	4.53±0.006	13c	6.86±0.00
54	14c	4.65±0.006	14c	6.24±1.08
55	15c	4.10±0.006	15c	6.48±0.00
56	16c	4.43±0.01	16c	6.59±0.00
57	17c	4.67±0.01	17c	6.33±1.08
58	18c	4.17±0.006	18c	6.89±0.00
59	19c	4.80±0.006	19c	6.76±1.08
60	20c	4.27±0.01	20c	6.54±0.00

3.3. Bacteria Endotoxin

The result of the Bacteria Endotoxin Test is as shown in the table below, five of the Pentazocine injections failed bacteria endotoxin test carried out on them. The results are 8.8, 8.9, 9.1, 9.4, and 8.8 against 8.7EU/ml British pharmacopeia limit. Two of the brands of the Diazepam injection failed BET at the range of 60 and 59 against 58.0 EU/ml British Pharmacopeia limit. The injections will course Rigor effect on the patients when used. This is a situation of high fever on the body of the patients. These results are similar to the result obtained by Ukwueze *et al* [18] in Port Harcourt Nigeria.

Table 2 Results of the Bacteria Endotoxin for Pentazocine and Diazepam Injections

S/N	Pentazocine Injection Brands	BP Specification (NMT8.7 EU/ml)		Diazepam Brands	BP Specification (NMT 58.0EU/ml)	
		Gel Clot Result	Chromogenic Result		Gel Clot Result	Chromogenic Result
1	1a	No Gel	0.1±0.06	1a	No Gel	30.2±0.21
2	2a	No Gel	0.01±0.00	2a	No Gel	29.3±0.85
3	3a	No Gel	0.01±0.00	3a	No Gel	17.3±11.43
4	4a	No Gel	1±0.00	4a	No Gel	20.17±0.07
5	5a	No Gel	0.2±0.06	5a	No Gel	25.16±5.25
6	6a	No Gel	0.1±0.06	6a	No Gel	10.11±0.050
7	7a	No Gel	0.01±0.00	7a	No Gel	0.11±0.005
8	8a	No Gel	0.3±0.01	8a	No Gel	0.052±0.001
9	9a	No Gel	0.30±0.02	9a	No Gel	4.1±0.050
10	10a	No Gel	0.2±0.02	10a	No Gel	2.62±0.007
11	11a	No Gel	0.1±0.03	11a	Gel Formed	60±0.000
12	12a	No Gel	0.04±0.00	12a	No Gel	1.00±0.001
13	13a	No Gel	0.5±0.006	13a	No Gel	2.20±0.050
14	14a	No Gel	1.0±0.12	14a	No Gel	1.91±0.001
15	15a	No Gel	0.01	15a	No Gel	25.50±0.000
16	16a	No Gel	3.0±0.00	16a	No Gel	19.40±0.001
17	17a	Gel Formed	8.8±0.02	17a	No Gel	0.53±0.001
18	18a	No Gel	0.01±0.00	18a	No Gel	32.0±0.050
19	19a	No Gel	0.4±0.02	19a	No Gel	9.22±0.001
20	20a	No Gel	0.11±0.00	20a	No Gel	2.12±0.002
21	1b	No Gel	0.30±0.00	1b	No Gel	4.31±0.050
22	2b	No Gel	3.1±0.00	2b	No Gel	4.21±0.002
23	3b	No Gel	0.2±3.40	3b	No Gel	5.54±0.050
24	4b	No Gel	0.5±0.20	4b	Gel Formed	59.04±0.000
25	5b	Gel Formed	8.9±0.00	5b	No Gel	2.11±0.001
26	6b	No Gel	0.9±0.00	6b	No Gel	2.30±0.005
27	7b	No Gel	0.1±0.31	7b	No Gel	6.41±0.005
28	8b	No Gel	0.05±8.50	8b	No Gel	9.81±0.000
29	9b	No Gel	0.8±1.36	9b	No Gel	0.35±0.001
30	10b	Gel Formed	9.1±0.00	10b	No Gel	2.22±0.001
31	11b	No Gel	0.3±0.01	11b	No Gel	0.12±0.000
32	12b	No Gel	0.08±0.00	12b	No Gel	9.01±0.000
33	13b	No Gel	0.11±0.00	13b	No Gel	3.21±0.050

34	14b	No Gel	0.2±3.40	14b	No Gel	1.41±0.001
35	15b	No Gel	0.8±0.01	15b	No Gel	2.51±0.002
36	16b	No Gel	0.6±0.03	16b	No Gel	6.64±0.001
37	17b	No Gel	0.09±1.70	17b	No Gel	40.19±0.001
38	18b	No Gel	8.0±0.00	18b	No Gel	2.24±0.050
39	19b	No Gel	2.0±0.00	19b	No Gel	25.21±0.001
40	20b	No Gel	0.06±0.00	20b	No Gel	0.11±0.00
41	1c	No Gel	0.4±0.01	1c	No Gel	8.27±0.001
42	2c	Gel Formed	9.4±0.00	2c	No Gel	3.33±0.00
43	3c	No Gel	0.5±0.00	3c	No Gel	3.61±0.00
44	4c	No Gel	0.2±3.40	4c	No Gel	3.23±0.005
45	5c	No Gel	0.7±1.36	5c	No Gel	0.45±0.012
46	6c	No Gel	0.01±0.00	6c	No Gel	1.55±0.001
47	7c	No Gel	0.5±0.01	7c	No Gel	3.98±0.00
48	8c	No Gel	4.2±0.00	8c	No Gel	0.43±0.003
49	9c	No Gel	0.91±0.00	9c	No Gel	3.47±0.00
50	10c	No Gel	0.08±0.00	10c	No Gel	1.75±0.00
51	11c	No Gel	3.9±0.00	11c	No Gel	0.14±0.001
52	12c	No Gel	0.2±3.40	12c	No Gel	0.34±0.002
53	13c	No Gel	0.2±3.40	13c	No Gel	8.73±0.01
54	14c	No Gel	0.6±0.00	14c	No Gel	4.82±0.000
55	15c	Gel Formed	8.8±0.00	15c	No Gel	7.10±0.001
56	16c	No Gel	1.2±0.01	16c	No Gel	5.44±0.001
57	17c	No Gel	2.0±0.00	17c	No Gel	6.65±0.001
58	18c	No Gel	0.02±0.00	18c	No Gel	6.73±0.000
59	19c	No Gel	0.4±6.80	19c	No Gel	8.69±0.001
60	20c	No Gel	1.0±0.00	20c	No Gel	4.71±0.001

3.4. Assay Results

The result of the Assays (Active Pharmaceutical Ingredients) in the results presented below shows that five of the brands failed assay test. Two brands are above specification at 105.13%, and 105.20 % against 105% upper limit. Three brands are below specifications at 94.67, 93.27 and, 94.93, against 95% lower limit. The implication is that drugs will not be able to treat the sickness that it is meant for because that will be an under dosage. Six of the Diazepam injections failed assay tests. Four are below the lower limit of the British pharmacopeia standard; they are at 86.20%, 83.80%, 87.60%, and 82.20%. Two of the samples were above the British pharmacopeia standard limit. The samples came at 114.00%, and 112.00%. These six brands will not be efficacious in human treatment; they will either be under dose or over dose in patients.

Table 3 Results of the Assay Results of the Pentazocine and Diazepam

S/N	Pentazocine Injection Brands	BP Specification Assay		Diazepam Brands	BP Specification Assay	
		In %w/v (1.425– 1.575)	In % (95 – 105)		In %w/v (0.450– 0.550)	In % (90 – 110)
1	1a	1.527±0.00	102.50±0.000	1a	0.510±0.002	102.00±0.310
2	2a	1.484±0.006	98.93±0.006	2a	0.550±0.006	110.00±0.105
3	3a	1.525±0.004	101.71±0.004	3a	0.490±0.001	98.00±0.012
4	4a	1.503±0.003	100.19±0.003	4a	0.470±0.001	94.00±0.013
5	5a	1.477±0.001	98.31±0.001	5a	0.530±0.001	106.00±0.012
6	6a	1.51±0.002	100.93±0.002	6a	0.451±0.003	90.20±0.003
7	7a	1.527±0.005	102.50±0.005	7a	0.463±0.010	92.60±0.010
8	8a	1.425±0.010	95.00±0.010	8a	0.515±0.004	103.00±0.004
9	9a	1.555±0.001	103.70±0.001	9a	0.455±0.021	91.00±0.021
10	10a	1.565±0. 010	104.33±0.010	10a	0.570±0.000	114.00±0.000
11	11a	1.577±0.020	105.13±0.020	11a	0.478±0.001	95.6.00±0.001
12	12a	1.575±0.100	105.00±0.100	12a	0.450±0.050	90.00±0.050
13	13a	1.562±0.030	104.13±0.030	13a	0.493±0.051	98.6.00±0.051
14	14a	1.487±0. 050	99.13±0. 050	14a	0.508±0.003	101.60±0.003
15	15a	1.566±0.001	104.40±0.001	15a	0.550±0.045	110.00±0.045
16	16a	1.578±0.002	105.20±0.002	16a	0.456±0.019	91.20±0.019
17	17a	1.574±0.002	104.93±0.002	17a	0.431±0.008	86.20±0.008
18	18a	1.420±0.004	94.67±0.004	18a	0.518±0.011	103.60±0.011
19	19a	1.544±0.051	102.93±0.051	19a	0.494±0.040	98.80±0.040
20	20a	1.468±0.020	97.86±0.020	20a	0.450±0.051	90±0.051
21	1b	1.425±0.003.	95.00±0.003	1b	0.490±0.016	98.00±0.016
22	2b	1.399±0.050	93.27±0.050	2b	0.530±0.000	106.00±0.000
23	3b	1.543±0.120	102.87±0.120	3b	0.481±0.118	96.20±0.118
24	4b	1.538±0.006	102.53±0.006	4b	0.525±0.101	105.00±0.101
25	5b	1.457±0.013	97.13±0.013	5b	0.451±0.002	90.20±0.002
26	6b	1.546±0.041	103.10±0.041	6b	0.512±0.113	102.40±0.113
27	7b	1.574±0.008	104.93±0.008	7b	0.520±0.010	104.00±0.010
28	8b	1.476±0.012	98.40±0.012	8b	0.541±0.005	108.20±0.005
29	9b	1.490±0.040	99.33±0.040	9b	0.549±0.018	109.80±0.018
30	10b	1.570±0.003	104.67±0.003	10b	0.419±0.006	83.80±0.006
31	11b	1.532±0.012	102.13±0.012	11b	0.531±0.016	106.20±0.016
32	12b	1.574±0.009	104.93±0.009	12b	0.522±0.002	104.40±0.002
33	13b	1.484±0.039	98.93±0.039	13b	0.468±0.010	93.60±0.010

34	14b	1.573±0.006	104.87±0.006	14b	0.500±0.011	100.00±0.011
35	15b	1.501±0.010	100.10±0.010	15b	0.499±0.003	99.80±0.003
36	16b	1.480±0.009	98.67±0.009	16b	0.540±0.041	108.00±0.041
37	17b	1.465±0.021	97.67±0.021	17b	0.550±0.019	110.00±0.019
38	18b	1.575±0.004	105.00±0.004	18b	0.457±0.007	91.40±0.007
39	19b	1.439±0.030	95.93±0.030	19b	0.466±0.011	93.20±0.011
40	20b	1.511±0.003	100.73±0.003	20b	0.560±0.005	112.00±0.005
41	1c	1.541±0.011	102.70±0.011	1c	0.477±0.019	95.40±0.019
42	2c	1.492±0.003	99.46±0.	2c	0.502±0.016	100.40±0.016
43	3c	1.464±0.050	97.60±0.	3c	0.462±0.012	92.40±0.012
44	4c	1.576±0.014	105.10±0.	4c	0.543±0.011	108.60±0.011
45	5c	1.424±0.007	94.93±0.	5c	0.438±0.022	87.60±0.022
46	6c	1.477±0.033	98.47±0.	6c	0.512±0.017	102.40±0.017
47	7c	1.479±0.005	98.60±0.	7c	0.504±0.003	100.80±0.003
48	8c	1.573±0.009	104.87±0.	8c	0.495±0.040	99.00±0.040
49	9c	1.548±0.000	103.20±0.	9c	0.513±0.015	102.60±0.015
50	10c	1.489±0.002	99.27±0.	10c	0.464±0.080	92.80±0.080
51	11c	1.460±0.001	97.33±0.	11c	0.531±0.071	106.20±0.071
52	12c	1.534±0.011	102, 00±0.	12c	0.510±0.015	102.00±0.015
53	13c	1.509±0.004	100.60±0.	13c	0.500±0.001	100.00±0.001
54	14c	1.575±0.051	105.00±0.	14c	0.411±0.002	82.20±0.002
55	15c	1.489±0.089	99.27±0.	15c	0.535±0.018	107.00±0.018
56	16c	1.471±0.007	98.10±0.	16c	0.510±0.001	102.00±0.012
57	17c	1.444±0.015	96.27±0.	17c	0.507±0.021	101.40±0.021
58	18c	1.542±0.008	102.80±0.	18c	0.549±0.016	109.80±0.016
59	19c	1.564±0.000	104.27±0.	19c	0.513±0.012	102.60±0.012
60	20c	1.458±0.041	97.20±0.	20c	0.493±0.005	98.60±0.005

Table 4 Results of the Particulate Matter Contamination for the Pentazocine and Diazepam Injections

S/N	**Pentazocine Injection Brands**	**BP Specification Particulate Contamination; Sub-Visible Particle 10µm ≤ 6000 25µm ≤ 600**	**Diazepam Brands**	**BP Specification Particulate Contamination; Sub-Visible Particle 10µm ≤ 6000 25µm ≤ 600**
1	1a	10µm = 0.50 25µm = 0.05	1a	10µm =1.70 25µm =0.55
2	2a	10µm = 0.60 25µm = 0.15	2a	10µm =0.40 25µm =0.10

3	3a	10µm = 1.25 25µm =0.05	3a	10µm = 2.70 25µm =1.55
4	4a	10µm = 1.55 25µm =0.05	4a	10µm =0.30 25µm =0.1p
5	5a	10µm = 0.05 25µm =0.10	5a	10µm = 0.76 25µm = 0.16
6	6a	10µm = 2.06 25µm =0.40	6a	10µm=0.20 25µm=0.00
7	7a	10µm =7.25 25µm =0.35	7a	10µm=0.40 25µm=0.00
8	8a	10µm=1.20 25µm=0.00	8a	10µm=0.00 25µm=0.00
9	9a	10µm=0.60 25µm=0.00	9a	10µm=1.40 25µm=0.40
10	10a	10µm=1.00 25µm=0.00	10a	10µm=1.70 25µm=0.55
11	11a	10µm=1.00 25µm=0.00	11a	10µm=0.40 25µm=0.00
12	12a	10µm=1.00 25µm=0.20	12a	10µm=0.40 25µm=0.20
13	13a	10µm=0.90 25µm=0.05	13a	10µm=0.80 25µm=0.00
14	14a	10µm=1.20 25µm=0.20	14a	10µm=0.40 25µm=0.20
15	15a	10µm=1.00 25µm=0.20	15a	10µm=0.40 25µm=0.10
16	16a	10µm=0.20 25µm=0.00	16a	10µm=0.00 25µm=0.00
17	17a	10µm=0.60 25µm=0.00	17a	10µm=0.80 25µm=0.00
18	18a	10µm=0.60 25µm=0.40	18a	10µm=5.80 25µm=3.40
19	19a	10µm=0.60 25µm=0.15	19a	10µm=3.20 25µm=2.40
20	20a	10µm=1.40 25µm=0.20	20a	10µm=1.00 25µm=0.40
21	1b	10µm=1.20 25µm=0.20	1b	10µm=2.70 25µm=1.55
22	2b	10µm=1.80 25µm=0.00	2b	10µm=0.40 25µm=0.00
23	3b	10µm=1.00 25µm=0.00	3b	10µm=0.40 25µm=0.00

24	4b	10µm=1.00 25µm=0.00	4b	10µm=0.40 25µm=0.00
25	5b	10µm=1.25 25µm=0.05	5b	10µm=0.40 25µm=0.20
26	6b	10µm=1.00 25µm=0.20	6b	10µm=0.20 25µm=0.00
27	7b	10µm=3.00 25µm=0.20	7b	10µm=0.20 25µm=0.00
28	8b	10µm=0.80 25µm=0.00	8b	10µm=0.30 25µm=0.10
29	9b	10µm=0.80 25µm=0.00	9b	10µm=1.60 25µm=0.00
30	10b	10µm=1.60 25µm=0.00	10b	10µm=1.60 25µm=0.40
31	11b	10µm=1.55 25µm=0.05	11b	10µm=2.80 25µm=0.40
32	12b	10µm=1.20 25µm=0.20	12b	10µm=1.40 25µm=0.20
33	13b	10µm=0.80 25µm=0.20	13b	10µm=0.80 25µm=0.20
34	14b	10µm=1.20 25µm=0.00	14b	10µm=0.80 25µm=0.00
35	15b	10µm=0.80 25µm=0.00	15b	10µm=1.65 25µm=0.25
36	16b	10µm=0.80 25µm=0.20	16b	10µm=1.00 25µm=0.00
37	17b	10µm=0.90 25µm=0.10	17b	10µm=0.80 25µm=0.00
38	18b	10µm=1.00 25µm=0.00	18b	10µm=0.00 25µm=0.00
39	19b	10µm=1.80 25µm=0.40	19b	10µm=3.20 25µm=1.20
40	20b	10µm=0.80 25µm=0.00	20b	10µm=1.60 25µm=1.00
41	1c	10µm=1.60 25µm=0.00	1c	10µm=1.45 25µm=0.55
42	2c	10µm=0.80 25µm=0.40	2c	10µm=1.15 25µm=0.25
43	3c	10µm=1.25 25µm=0.20	3c	10µm=1.60 25µm=0.00
44	4c	10µm=0.00 25µm=0.00	4c	10µm=0.40 25µm=0.00

45	5c	10µm=0.40 25µm=0.00	5c	10µm=0.20 25µm=0.00
46	6c	10µm=0.80 25µm=0.20	6c	10µm=0.80 25µm=0.20
47	7c	10µm=0.40 25µm=0.00	7c	10µm=0.80 25µm=0.20
48	8c	10µm=0.60 25µm=0.20	8c	10µm=0.55 25µm=0.10
49	9c	10µm=0.55 25µm=0.10	9c	10µm=0.00 25µm=0.00
50	10c	10µm=1.60 25µm=0.00	10c	10µm=0.40 25µm=0.00
51	11c	10µm=0.40 25µm=0.00	11c	10µm=0.80 25µm=0.20
52	12c	10µm=0.20 25µm=0.00	12c	10µm=0.40 25µm=0.00
53	13c	10µm=0.80 25µm=0.20	13c	10µm=0.60 25µm=0.20
54	14c	10µm=0.80 25µm=0.20	14c	10µm=0.55 25µm=0.10
55	15c	10µm=0.55 25µm=0.10	15c	10µm=1.20 25µm=0.20
56	16c	10µm=10.60 25µm=7.40	16c	10µm=1.00 25µm=0.20
57	17c	10µm=2.20 25µm=0.60	17c	10µm=0.80 25µm=0.00
58	18c	10µm=0.60 25µm=0.00	18c	10µm=1.60 25µm=0.00
59	19c	10µm=0.40 25µm=0.40	19c	10µm=1.80 25µm=0.40
60	20c	10µm=1.40 25µm=0.00	20c	10µm=1.00 25µm=0.00

3.5. Volume Results

The volume measurements are all within the specifications.

Table 5 Results of theVolume check for the Pentazocine and Diazepam Injections

S/N	Pentazocine Injection Brands	BP Specification (2.0 - 2.1)	Diazepam Brands	BP Specification (2.0 - 2.1)
1	1a	2.0	1a	2.0
2	2a	2.0	2a	2.0
3	3a	2.0	3a	2.0
4	4a	2.0	4a	2.0
5	5a	2.0	5a	2.0
6	6a	2.0	6a	2.0
7	7a	2.0	7a	2.0
8	8a	2.0	8a	2.0
9	9a	2.0	9a	2.0
10	10a	2.0	10a	2.0
11	11a	2.0	11a	2.0
12	12a	2.0	12a	2.0
13	13a	2.0	13a	2.0
14	14a	2.0	14a	2.0
15	15a	2.0	15a	2.0
16	16a	2.0	16a	2.0
17	17a	2.0	17a	2.0
18	18a	2.0	18a	2.0
19	19a	2.0	19a	2.0
20	20a	2.0	20a	2.0
21	1b	2.0	1b	2.0
22	2b	2.0	2b	2.0
23	3b	2.0	3b	2.0
24	4b	2.0	4b	2.0
25	5b	2.0	5b	2.0
26	6b	2.0	6b	2.0
27	7b	2.0	7b	2.0
28	8b	2.0	8b	2.0
29	9b	2.0	9b	2.0
30	10b	2.0	10b	2.0
31	11b	2.0	11b	2.0
32	12b	2.1	12b	2.0

33	13b	2.0	13b	2.0
34	14b	2.0	14b	2.0
35	15b	2.0	15b	2.0
36	16b	2.0	16b	2.0
37	17b	2.0	17b	2.0
38	18b	2.0	18b	2.0
39	19b	2.0	19b	2.0
40	20b	2.0	20b	2.0
41	1c	2.0	1c	2.0
42	2c	2.0	2c	2.0
43	3c	2.0	3c	2.0
44	4c	2.0	4c	2.0
45	5c	2.0	5c	2.0
46	6c	2.0	6c	2.0
47	7c	2.0	7c	2.0
48	8c	2.0	8c	2.0
49	9c	2.0	9c	2.0
50	10c	2.0	10c	2.0
51	11c	2.0	11c	2.0
52	12c	2.0	12c	2.0
53	13c	2.0	13c	2.0
54	14c	2.0	14c	2.0
55	15c	2.0	15c	2.0
56	16c	2.0	16c	2.0
57	17c	2.1	17c	2.0
58	18c	2.0	18c	2.0
59	19c	2.0	19c	2.0
60	20c	2.1	20c	2.0

3.6. Sterility Result.

All the samples passed the sterility tests. This shows that the samples have no aerobic and anaerobic organisms in them. The fluid thioglacolate media and the soya bean casein digest media used for the tests were initially tested for viability and they all passed viability test to be able to detect and grow the organisms. Hence the samples are fit for the purpose sterility wise.

Table 6 Results of the Sterility check for the Pentazocine and Diazepam Injections

S/N	Pentazocine Injection Brands	Sterility (Must be sterile)	Diazepam Injection Brands	Sterility (Must be sterile)
1	1a	Sterility	1a	Sterility
2	2a	Sterility	2a	Sterility
3	3a	Sterility	3a	Sterility
4	4a	Sterility	4a	Sterility
5	5a	Sterility	5a	Sterility
6	6a	Sterility	6a	Sterility
7	7a	Sterility	7a	Sterility
8	8a	Sterility	8a	Sterility
9	9a	Sterility	9a	Sterility
10	10a	Sterility	10a	Sterility
11	11a	Sterility	11a	Sterility
12	12a	Sterility	12a	Sterility
13	13a	Sterility	13a	Sterility
14	14a	Sterility	14a	Sterility
15	15a	Sterility	15a	Sterility
16	16a	Sterility	16a	Sterility
17	17a	Sterility	17a	Sterility
18	18a	Sterility	18a	Sterility
19	19a	Sterility	19a	Sterility
20	20a	Sterility	20a	Sterility
21	1b	Sterility	1b	Sterility
22	2b	Sterility	2b	Sterility
23	3b	Sterility	3b	Sterility
24	4b	Sterility	4b	Sterility
25	5b	Sterility	5b	Sterility
26	6b	Sterility	6b	Sterility
27	7b	Sterility	7b	Sterility
28	8b	Sterility	8b	Sterility
29	9b	Sterility	9b	Sterility
30	10b	Sterility	10b	Sterility
31	11b	Sterility	11b	Sterility
32	12b	Sterility	12b	Sterility
33	13b	Sterility	13b	Sterility
34	14b	Sterility	14b	Sterility

35	15b	Sterility	15b	Sterility
36	16b	Sterility	16b	Sterility
37	17b	Sterility	17b	Sterility
38	18b	Sterility	18b	Sterility
9	19b	Sterility	19b	Sterility
40	20b	Sterility	20b	Sterility
41	1c	Sterility	1c	Sterility
42	2c	Sterility	2c	Sterility
43	3c	Sterility	3c	Sterility
44	4c	Sterility	4c	Sterility
45	5c	Sterility	5c	Sterility
46	6c	Sterility	6c	Sterility
47	7c	Sterility	7c	Sterility
48	8c	Sterility	8c	Sterility
49	9c	Sterility	9c	Sterility
50	10c	Sterility	10c	Sterility
51	11c	Sterility	11c	Sterility
52	12c	Sterility	12c	Sterility
53	13c	Sterility	13c	Sterility
54	14c	Sterility	14c	Sterility
55	15c	Sterility	15c	Sterility
56	16c	Sterility	16c	Sterility
57	17c	Sterility	17c	Sterility
58	18c	Sterility	18c	Sterility
59	19c	Sterility	19c	Sterility
60	20c	Sterility	20c	Sterility

4. Conclusion

The quality of Pentazocine injection that are in circulation in Anambra State shows that many of the brands have good quality as shown from the result in the tables above, however there are still much room for improvement on some of the brands in circulation as can be seen from the results of the assay where some of the brands are very close to the lower limit of the assay specification. The quality of Diazepam injections in circulation in Anambra State shows that there are poor quality Diazepam in the State and this calls for serious regulatory surveillance on the quality of injections that are in circulation in the state. If poor quality drugs are allowed to be in circulation in the state, it will affect the workforce of the populace of the state and thereby affect the economy of the state. The health status of the dwellers in the state will be in jeopardy if substandard drugs are allowed to continue to circulate in the state. The issue of drug resistance and other risks associated with low quality drugs are on the increase in several places round the globe and could be reduced by insisting that only quality assured medicines comes into the state.

Compliance with ethical standards

Acknowledgments

Our sincere gratitude goes to the Almighty God for His mercy, guidance and protection throughout this research. Our gratitude goes to the companies that released to us their products for used for this study. We appreciate in a special way the laboratories that permitted us to carry out the different analysis in their laboratories.

Disclosure of conflict of interest

The Authors declares that there is no conflict of interest.

References

[1] Achermann, J.C., and Jameson, J.L. (1999), Fertility and infertility: genetic contributions from the hypothalamic-pituitary-gonadal axis. Moi. Endocrinol., 13:812-818.

[2] AnneLoes van Staa & Anita Hardon (2000).Injection practices in the developing world Results and recommendations from field studies in Uganda and Indonesia. Medical Anthropology Unit University of Amsterdam Oudezijds Achterburgwal 185 1012 DK Amsterdam the Netherlands.

[3] Calcaterra NE, Barrow JC. Classics in chemical neuroscience: diazepam (valium). ACS Chem Neurosci. 2014 Apr 16, 5(4):253-60. [PMC free article] [PubMed].

[4] Pharm Access foundation (2015).Nigerian Health Sector Market Study Report, Study commissioned by the Embassy of the Kingdom of the Netherlands in Nigeria.

[5] SatishVarpe, Laxman Gadekar, Rahul Kunkulol (2016). Evaluation of Pentazocine in Acute Myocardial Infarction.National Journal of Medical and Dental Research, Volume-4, Issue-2, Page 135-139

[6] Harold .M. Walker and Paul .A. Torres (2014). Diazepam Medical uses, Pharmacology and Health effect. Published by Nova Science Publishers Inc.New York United States of American.ISBN: 978-1-62948-628-4.

[7] Iheanacho O.E, Ezenwenyi I.P, and Enosolease M.E (2015). Pentazocine abuse in Sickle Cell Disease Patients Seen at a Tertiary Hospital in Nigeria: A Chronic Menace. International Journal of TROPICAL DISEASE& Health 9(1): 1-8, Article no.IJTDH.18336ISSN: 2278–1005SCIENCEDOMAIN international. www.sciencedomain.org.

[8] Madu AJ, Korubo K, Okoye H, Ugwu, Efobi, Nwogoh and Okoye (2018). Survey of Pentazocine Addiction and Opioid Use in Adult Sickle Cell Anaemia Patients: The Perspective of Healthcare Providers. Haematology International Journal ISSN: 2578-501X.

[9] Ademola Adewoyin, &, Oluwafemi Adeyemi, Nosimot Davies, Matilda Ojo (2019). Clinical and socio-demographic determinants of pentazocine misuseamong patients with sickle cell disease, Benin City, Nigeria: a case control study. Pan African Medical Journal.2019, 34:88. doi:10.11604/pamj.2019.34.88.17257. http://www.panafrican-med-journal.com/content/article/34/88/full/

[10] Cannizzaro, E., M. Martire, M. Gagliano, F. Plescia, M. La Barbera, G. Mantia, A. Mine0, G. Cannizzaro and C. Cannizzaro, 2005.Reversal of prenatal diazepam-induced deficit in a spatial-object learning task by brief, periodic maternal separation in adult rats.Behav.Brian., 161:320-330.

[11] European Medicines Agency(2000). Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances (CPMP/ICH/367/96).

[12] PranithaAkula Lakshmi P.K(2018)Effect of pH on weakly acidic and basic model drugs and determination of their ex vivo transdermal permeation routes.

[13] UDEGBUNAM, Rita Ijeoma, UDEGBUNAM, Sunday Ositadimma, ONUBA, Austine Chukwudum, UGWU, Nnenna Ebere (2017). Evaluation Of The Use Of Pentazocine In Combination With Diazepam And Ketamine For Surgical Anaesthesia In Rabbits. Animal Research International (2017) 14(1): 2562 – 2567.

[14] Olike Chinwendu (2008). The fight against fake drugs by NAFDAC in Nigeria. 44th International Course in Health Development (ICHD) September 24, 2007 – September 12, 2008. KIT (Royal Tropical Institute) Development Policy & Practice/Vrije Universiteit Amsterdam.

[15] G. H. Tan,L. W. Dittert,J. T. Doluisio (2011). Drug absorption IV: Influence of pH on absorption kinetics of weakly acidic drugs.

[16] Harold .M. Walker and Paul .A. Torres.(2015) Diazepam Medical uses, Pharmacology and Health effect. Published by Nova Science Publishers Inc.New York United States of American.ISBN: 978-1-62948-628-4.

[17] Chioma Joy Ebenezer (2015). Pharmaceutical Quality And Policy In Nigeria: Stakeholder Perspectives And Validation Of The Mobile AuthenticationService. Department of Pharmacy, University College London.

[18] ICH guideline Q3D (R1) on elemental impurities (2019).Committee for Human Medicinal Products.

[19] Jose Benjamin (2005). In what circumstances HPLC assay is more than 100%.

[20] Klaus T. Olkkola, Timo Seppäiä, Eeva-Lüsa Maunuksela (1993). Pharmacokinetics and Pharmacodynamics of Pentazocine in Children.First published: https://doi.org/10.1111/j.1600-0773.1993.tb01547.

[21] Ku LC, Hornik CP, Beechinor RJ, Chamberlain JM, Guptill JT, Harper B, Capparelli EV, Martz Koji Yokoyama 1, Naoto Takahashi, Yukari Yada, Yasunori Koike, RyouKawamata, RiteiUehara, YumiKono, Yoko Honma, Mariko Y Momoi (2010). Prolonged maternal magnesium administration and bone metabolism in neonates.

[22] Lawrence O. Gostin, Daniel Carpenter, Hans Hogerzeil, Thomas Layloff, Patrick Lukulay, Ann Marie Kimball, MargarethNdomondo-Sigonda, ArtiRai, Marco Antonio Stephano, John Theriault, Mary Wilson, PrashantYadav. (2013) Committee on Understanding the Global Public Health Implications of Substandard, Falsified, and Counterfeit Medical Products.Institute of Medicine of the national academies.

[23] K, Anand R, Cohen-Wolkowiez M, Gonzalez D (2018)., Best Pharmaceuticals for Children Act - Pediatric Trials Network Steering Committee. Population Pharmacokinetics and Exploratory Exposure-Response Relationships of Diazepam in Children Treated for Status Epilepticus. CPT PharmacometricsSystPharmacol. Nov, 7(11):718-727. [PMC free article] [PubMed]

[24] K. Rifat, Alastair A. Spence and. J C. Davis(1972).PentazocineIn Sequential Analgesic Anaesthesia

[25] Lakshmi Prasanna K (2017) Sterility Assurance of Parenteral Products–Major Deficiency for Recall. J Pharmacovigil 5: e168. doi: 10.4172/2329-6887.1000e168

[26] Laura Christian, Linda Collins, MaythiwanKiatgrajai, Agathe Merle, NayantaraMukherji, AlissaQuade (2012). The Problem of Substandard Medicines in Developing Countries.

[27] Lawrence O. Gostin, Daniel Carpenter, MargretthNdomondo-Signada, ArtiRai (2013). Countering the Problem of Falsified and Substandard Drugs.

[28] Lawrence A.T., (2009) Handbook on Injectable Drugs 15th Edition, .American Society of Health-System Pharmacists. Printed on 4t0#Domitar Westminster Opaque FSC certified. 10% PCW content. ISBN:978-1-58528-213-5. Pp 188

[29] Mandelli M, Tognoni G, Garattini S. (1978), Clinical pharmacokinetics of diazepam. ClinPharmacokinet. 3(1):72-91. [PubMed]

[30] Mick Dawson (2017) ENDOTOXIN LIMITS For Parenteral Drug Products, BET Whi t e Paper vol .1 no. 2.Associates of Cape Cod, Inc., East Falmouth, MA 02536 ~ 508.540.3444.www.acciusa.com PR# 17-012

[31] NasrinTakazare, Mir-Jamal Hosseini, AlirezaTakzare, AzamBakhtiarian, Kamran Yarmohammadi. (2008). Teratogenic Effect of Diazepam Intake During Pregnancy Leading to Cleft Lip and Palatal Anomalies.

[32] Karen Henderson (2008). Update in Anaesthesia. Education for anesthetists worldwide. The Journal of the World Federation of Societies of Anaesthesiologists Volume 24 Number 1 ISSN 1353-4882 www.worldanaesthesia.org

[33] S.P.I. Ogah, E. E. Ukaegbu (2019). Physicochemical analysis of water samples from Lafia metropolis, Nasarawa State, Nigeria. IOSR Journal of Applied Chemistry (IOSR-JAC) e-ISSN: 2278-5736.Volume 12, Issue 8 Ser. II (August. 2019), PP 13-23 www.iosrjournals.org.

[34] Stanley E. Ukwueze, Constance G. Okpalaeke, and Olusayo A. Shorinwa (2015). Physicochemical and Microbiological assessment of some commercially available Sterile Water for Injection brands in Nigeria. World Journal of Pharmaceutical Research. Volume 4, Issue 6, 186-196. ISSN 2277-7105.

Author's short biography

	**Ezugwu John Afamefuna** He is a PhD student of Analytical Chemistry in Chukwuemeka Odumegwu Ojukwu University (Former Anambra State University), Anambra State. He has MSc.in Analytical Chemistry from Chukwuemeka Odumegwu Ojukwu Anambra State. He has BSc in Industrial Chemistry from Enugu State University of Technology Enugu. Area of interest in teaching and research in the area of Chemistry and its relationship to Pharmaceutical Technology and Drug development. Also in chemistry and its application to nano-technology in natural and synthetic drug development.
	**Sylvia Ifeyinwa Okonkwo** She is a professor of Analytical Chemistry in Chukwuemeka Odumegwu Ojukwu University (Former Anambra State University) Anambra State. Has PhD in analytical chemistry from Federal University of Technology Oweri. Obtained MSc. in Chemical Technology from Nnamdi Azikiwe University Awka. Has BSc. In Industrial Chemistry from Nnamdi Azikiwe University Awka. Area of interest is in teaching and research in the area of Chemistry and its application to nano-technology such as using metabolite from natural product to synthesized nano particles and nano emulsion for pharmaceutical purposes.
	**Ezugwu Edith Chinyere** She is a Technologist in Biochemistry Department Nnamdi Azikiwe University Awka Anambra State. She obtained Post Graduate Diploma in education from National Open University of Nigeria. She has HND from Yaba College of Technology Lagos, Lagos State. She has interest in teaching and researching in the area of biochemical and synthesis of drugs for maternal health.
	**Charles Kenechukwu Okonkwo MBBS** He completed his bachelor of medicine bachelor of surgery at Chukwuemeka Odumegwu Ojukwu University School of Medicine Awka, Anambra State. Completed his One Year Internship at General Hospital Onitsha, Anambra State. His research interests include allergy and immunology, sports medicine, primary and critical care medicine, and rehabilitative and physical therapy.
	**Chinwe C Okonkwo, MD, RVT** She completed Medical Doctor Degree from Caribbean Medical University School of Medicine Curaçao Registered Vascular Technologist with American Registry for Diagnostic Medical Sonography Bachelor's Degree in Biotechnology Nnamdi Azikiwe University Department of Science Technology. Her Research interests include Clinical and Health service research, Primary care and community Medicine and Point of Care Ultrasound.