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Full Length Research Paper

Verification of pharmaceutical power of generic meropenem vs innovator trough assessment by microbial potency test

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This study presents the development of a microbiological test method with different microorganisms and different concentrations of antibiotics through "microbial potency," which established the dose-response concentrations and test conditions. The assay method was based on the plate cylinder with the result that there is equivalence between the pharmaceuticals antibiotic such as generic meropenem and innovator for the microorganisms such as *Bacillus subtilli* ATCC6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619 and *Escherichia coli* ATCC 10536, and pharmaceutical equivalence does not exist between the generic antibiotic meropenem and innovative meronem for *Microococcus luteus* ATCC 9341.

Key words: Microbial power, cylindrical methodin plate, antibiotics, pharmaceutical equivalence, *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, *Microococcus luteus* ATCC 9341.

INTRODUCTION

The market for medicines has been dramatically influenced by two major segments, innovative medicines which contain an innovative active ingredient, as a result of extensive research and backed by a patent, on the other side are generic drugs which according to the World Health Organisation/ Pan American Health Organisation (WHO/PAHO) are defined as follows: "Two products are pharmaceutically equivalent if they contain the same amount of the same substance or active ingredient, in the same pharmaceutical modality, if they have identical or comparable standards and if they are suitable for administration by the same route and same directions, if meet the same or comparable quality standards, although they have differences in the excipients or ways of processing" (WHO, 2011). A generic drug is the same as a brand name drug in dosage, safety, strength, how it is taken, quality, performance, and intended use. Before approving a generic drug product, the Food and Drug Administration (FDA) requires many rigorous tests and procedures to assure that the generic drug can be substituted for the brand

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name drug. The FDA bases evaluations of substitutability, or "therapeutic equivalence," of generic drugs on scientific evaluations. By law, a generic drug product must contain the identical amounts of the same active ingredient(s) as the brand name product. Drug products evaluated as "therapeutically equivalent" can be expected to have equal effect and no difference when substituted for the brand name product (USP 29, 2006).

It is believed that generic drugs are less expensive because they do not have the same benefits and efficiency as the innovator. Therefore it is important to determine and demonstrate the pharmaceutical equivalence between generic medications and the innovator, and thus to be sure of the quality in the use of these medications since the consequence can lead to serious health problems of patients if they do not have the same active principle and therefore do not act the same way. Meropenem is an antibiotic that has a broad spectrum of bacteria and is used to treat serious respiratory infections, nosocomial infections, intraabdominal infections, urinary tract infections, meningitis, septicemia, pediatric, gynecological infections, monotherapy in immunocompromised patients and others; it is marketed as an innovator product and as a generic product. This research will verify the pharmaceutical equivalence of the generic meropenem versus the innovator through microbial potency on the microbial strains of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29737, *Pseudomonas aeruginosa* ATCC 25619, *Escherichia coli* ATCC 10536, and *Microococcus luteus* ATCC 9341, using the turbidimetric method, which will allow us to know the effect on the previously appointed microorganisms and if in fact there is pharmaceutical equivalence between the innovator meropenem and the generic meropenem (Alvarez, 2008).

MATERIALS AND METHODS

For this study, the standardization of the antibiotic means used for the power tests and the antibiotic concentrations was performed.

Inoculum preparation

For this study, the microorganisms *B. subtilis* ATCC 6633, *E. coli* ATCC10536, *M. luteus* ATCC 9341, *P. aeruginosa* ATCC 25619 and *S. aureus* ATCC 29737 were used, in accordance with the USP Pharmacopeia; which underwent 4 chimes of each strain in trypticase soy agar (TSA) agar, these organisms were submitted to analysis when they were in exponential phase; the organism suspension was prepared in sterile saline 0.9% at a transmittance of 25% + / - 0.5 to 600 nm.

Preparation and inoculation of the plate

20 ml of medium antibiotic and 0.6 ml of organism were served under sterile conditions. The solution was stirred to obtain a homogeneous solution, then it was allowed to solidify. In each box, 6 reservoirs were worked and 3 were for the S3 standard and the remaining three were for the corresponding sample and other standards as the case may be.

Preparation of the standard solution

A USP standard solution and a VICAR secondary standard, for which a weight of 36.97 and 34.53 mg respectively, was calculated in a 50 mL volumetric balloon and it was completed on volume with a phosphate buffer of pH 8, therefore, 5 ml in a 50 ml volumetric balloon were taken and was completed with phosphate buffer of pH 8, consequently, the concentrations in 0.2, 0.4, 0.8, 1.6 and 3.2 ug/ml were performed.

Sample preparation

For this study, the following tests were performed: Generic antibiotic of Meropenem 1g and 05 vs. USP standard and VICAR secondary standard; Meropenem 1 g innovator antibiotic and 0.5 vs. USP standard and VICAR secondary standard; meropenem 29.35 mg generic antibiotic and meronem 30 mg innovator antibiotic, each in volumetric balloon of 50 mL and was completed with a phosphate buffer of pH 8. Consequently, 5 ml were taken in volumetric balloon of 50 ml and it was completed with phosphate buffer pH 8. Consequently, the 0.2, 0.4, 0.8, 1.6 and 3.2 ug/ml concentrations were performed.

Procedure for the essay through the cylinder-plate method

Boxes were placed for the analysis on a level surface. 20 mL of antibiotic medium inoculated (0.3 ml per 20 ml of medium) were served in each box and were allowed to solidify. In each box, 6 reservoirs were worked of which 3 were for the standard S3 and the other three corresponded to the sample and other standards as applicable, each cylinder with 0.1 ml sample of the antibiotic. The plates were incubated 16-18 h at 37°C. After incubation time, the cylinders were removed from the agar and the diameters of the inhibition zones were measured (Kenneth, 1994).

Statistical design

A variance analysis was conducted on two factors with several samples per group to verify the pharmaceutical equivalence between generic meropenem and the innovator, compared with USP reference standard and secondary VICAR standard. The study posed the following three hypotheses:

First hypothesis

Ho: (null hypothesis). There is no pharmaceutical equivalence between the meropenem generic antibiotic and meronem.
Ha: (alternative hypothesis) There is pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator.

Second hypothesis

Ho: The concentrations do not have effect on the diameter.
Ha: The concentrations have effect on the diameter.

Third hypothesis

Ho: There is a joint effect between the pharmaceutical equivalence of the antibiotics and the concentrations.
Ha: There is no joint effect between the pharmaceutical equivalence of the antibiotics and the concentrations.

The following probability was formulated: If $p \leq 0.05$, rejection of the Ho; If $p \geq 0.05$, acceptance of the Ho.

Table 1. Inhibition halos in Antibiotic media N° 1 and 11.

Microorganism	Antibiotic media No. 1	Antibiotic media No. 11	Ideal media
Staphylococcus aureus	Diffused and irregular halos	Defined and regular halos	Antibiotic media No. 11
Micrococcus luteus	Diffused and irregular halos	Diffused and irregular halos	Antibiotic media No. 1
Pseudomonas aeruginosa	Defined and regular halos	Diffused and irregular halos	Antibiotic media No. 1
Bacillus subtillis	Defined and regular halos	Diffused and irregular halos	Antibiotic media
Escherichia coli	Defined and regular halos	Diffused and irregular halos	Antibiotic media

Table 2. R^2 of the different microorganisms.

Microorganism	R^2 of each microorganism					
	Standard USP			Secondary standard		
	Meropenem	Meronem	Standard USP	Meropenem	Meronem	Secondary standard
Bacillus subtillis	0.99	0.98	0.98	0.98	0.98	0.98
Micrococcus luteus	0.98	0.98	0.99	0.99	0.99	0.99
Staphylococcus aureus	0.99	0.98	0.98	0.98	0.98	0.95
Pseudomona aeruginosa	0.98	0.98	0.99	0.98	0.99	0.98
Escherichia coli	0.99	0.99	0.98	0.98	0.98	0.99

RESULTS

The ideal media for growth of each microorganism was chosen, where a neat halo and regular growth was clearly shown (Table 1).

Determination of standard curve

For this study was considered two presentations for generic antibiotic and two presentations for these being innovative antibiotic 1 g and 0.5 mg, and prepared two curves: one standard and one standard USP secondary. Once the experiments were concluded by the standard deviation of antibiotics presentations 1 g and 0.5 mg yield the same results, so we proceeded to use for the entire study presentation of 1 g antibiotics. From the measurement of growth inhibition zones, averages were taken with antibiotic concentrations used and tracing the curve for each of the microorganisms from this equation was obtained from the subtraction and the coefficient of determination (r2) (Morales, 1957).

The r2 obtained for each of the curves studied both as the standard samples gave data ≥ 0.98 (Table 2) which fall within the acceptance range, showing a relationship between the values of both shafts (Fernandez et al 2006). By plotting standard curves of USP meropenem 1 g and meronem against microorganisms *B. subtilis*, *M. luteus*, *E. coli*, *P. aeruginosa*. (Figures 6, 7, 9 and 10), it appears that the stock is the left side of the standard, which means that the power stated is correct, however the values of *S. aureus* are located on the right side of the standard curve so the stated concentrations is not successful (Figure 8). The r^2 obtained for each of the studied curves both for standards and figures showed data of ≥0.98.

In the curve graphics of the VICAR secondary standard, the meropenem and meronem 1 g showed values below the standard for microorganisms such as *B. subtillis, M. luteus, S. aureus, E. coli* and *P. aeruginosa*. The averages obtained from the power percentages of all microorganisms, compared to the standard secondary curve were greater or equal to the amount labeled for the generic antibiotic and the innovator (Table 3). There is a pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator for the *B. subtillis, S. aureus, P. aeruginosa* and *E. coli* microorganisms. There is no pharmaceutical equivalence between the meropenem generic antibiotic and the meronem innovator on the *M. luteus* microorganism.

Statistical analysis

In the first case, the null hypothesis was rejected for allmicroorganisms except for *M. luteus*. In the second case the null hypothesis was rejected for all microorganisms. In the third case the null hypothesis was rejected for all microorganisms except for *M. luteus* (Table 4). In the first case the null hypothesis was rejected for all the microorganisms except for *E. coli.* In the second case the null hypothesis was rejected for all microorganisms. In the third case, the null hypothesis was rejected for all microorganisms except for *E. coli* (Table 5).

Table 3. Power percentage for each microorganism.

Microorganism	Potency percentage			
	Standard USP		Secondary standard	
	Meropenem	Meronem	Meropenem	Meronem
Bacillus subtillis	78.18	78.47	90.1	92.1
Micrococcus luteus	86.87	86.87	85.1	89.1
Staphylococcus aureus	81.27	81.27	84.2	82.5
Pseudomona aeruginosa	80.38	88.14	82.1	91.3
Escherichia coli	91.97	95.72	86.5	86.5

Table 4. USP standard probabilities.

Microorganism	Probability		
	First hypothesis	Second hypothesis	Third hypothesis
Bacillus subtillis	5.24871E-25	5.7789E-123	2.56773E-50
Micrococcus luteus	0.999996725	1.2183E-118	0.999146312
Staphylococcus aureus	1.22157E-38	1.4671E-128	4.74343E-30
Pseudomonas aeruginosa	3.69496E-74	2.8246E-134	2.0361E-56
Escherichia coli	8.24422E-54	4.5048E-138	6.34906E-58

Table 5. Secondary standard probabilities.

Microorganism	Probability		
	First hypothesis	Second hypothesis	Third hypothesis
Bacillus subtillis	8.14039e-40	1.6195e-124	3.5956e-31
Micrococcus luteus	1.23425e-50	1.9309e-120	2.98384e-68
Staphylococcus aureus	3.85579e-14	1.043e-125	1.23678e-44
Pseudomonas aeruginosa	2.92389e-58	2.1076e-126	5.67466e-52
Esherichia coli	0.486602146	1.152E-118	0.621385479

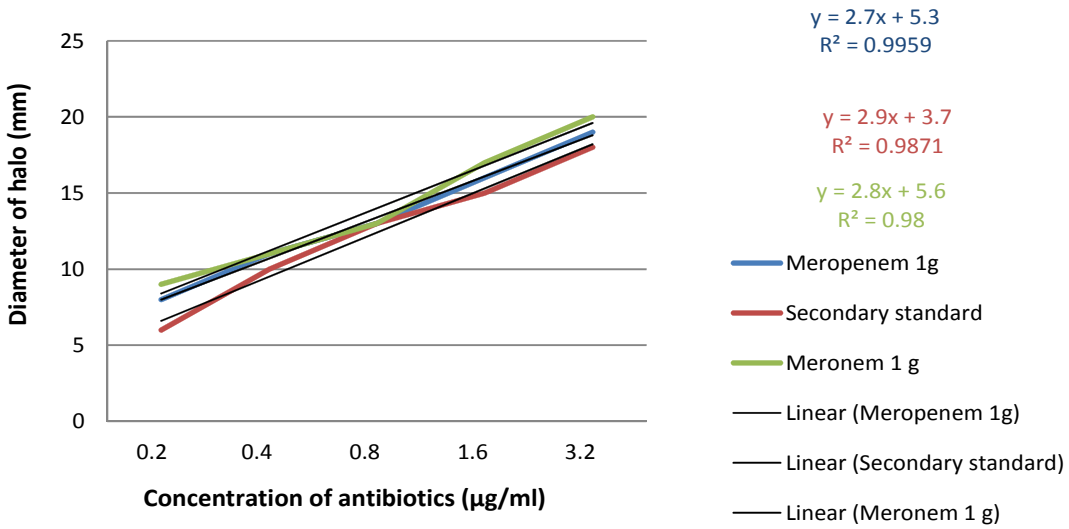

Figure 1. Secondary standar curve , meropenemand meronem with *Bacillus subtillis*.

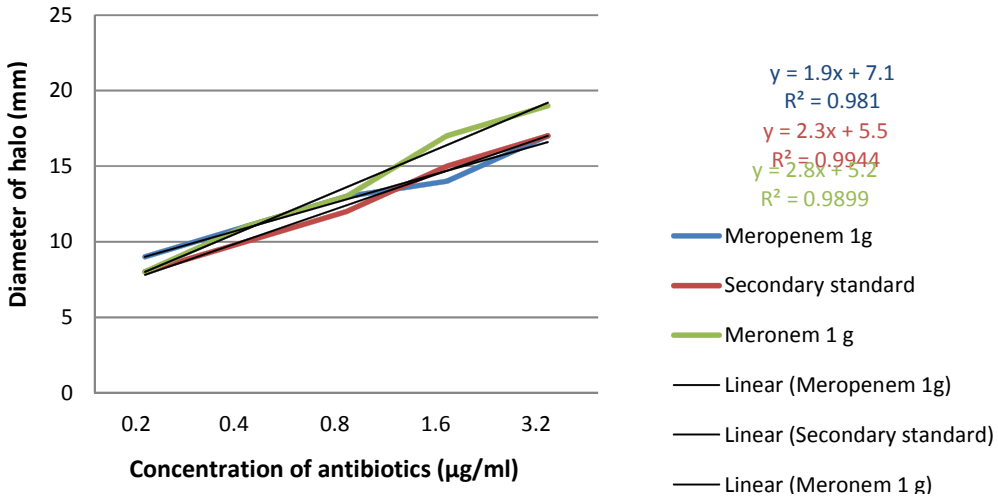

Figure 2. Secondary standard curve of meropenem and meronem with *Micrococcus luteus*.

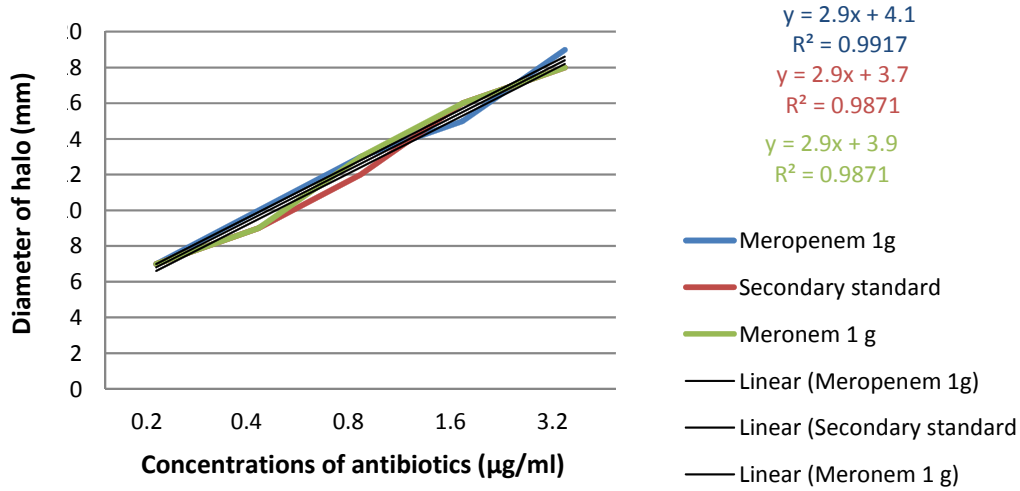

Figure 3. Secondary stand curve, meropenem and meronem with *Staphylococcus aures.*

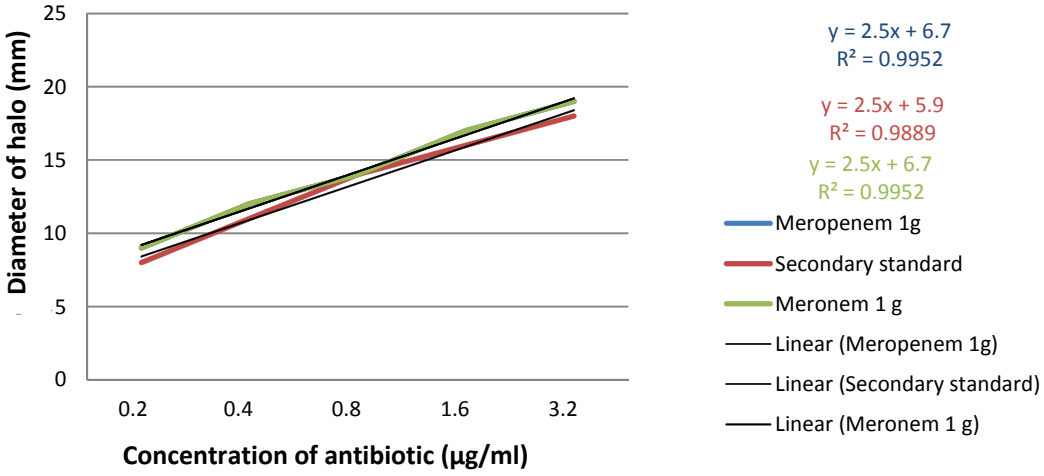

Figure 4. Secondary stándar curve VICAR , meropenem and meronem with *E. coli*.

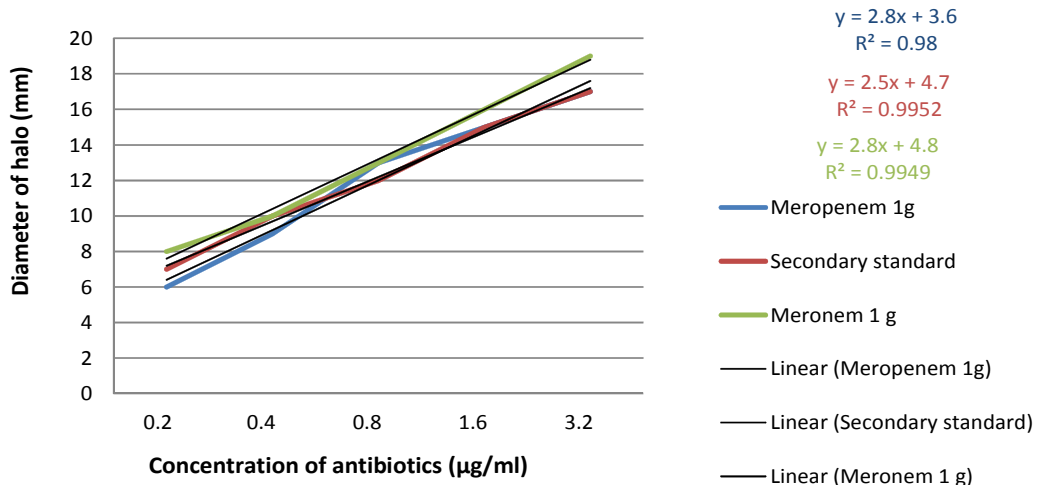

Figure 5. Secondary stándar curve VICAR, meropenem and meronem with *Pseudomona aeruginosa*.

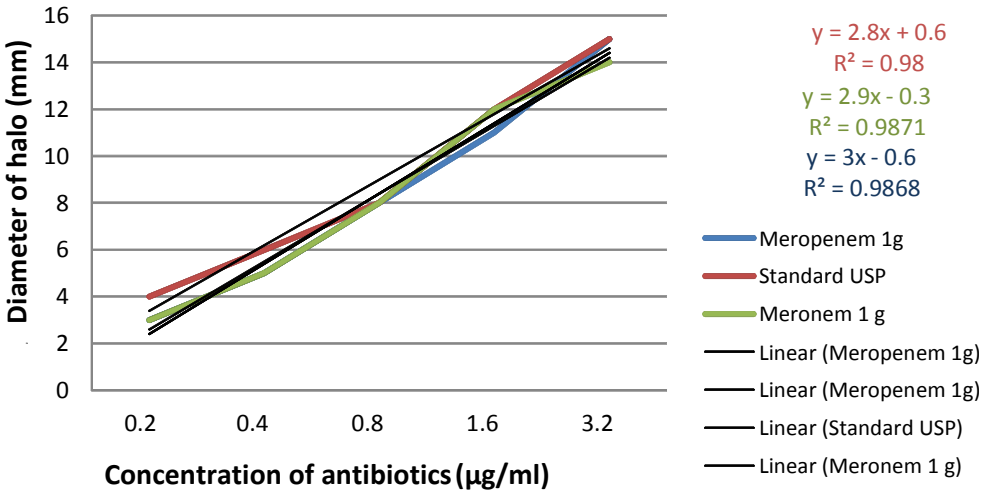

Figure 6. USP standard curve, Meropenem andMeronem with *Bacillus subtillis.*

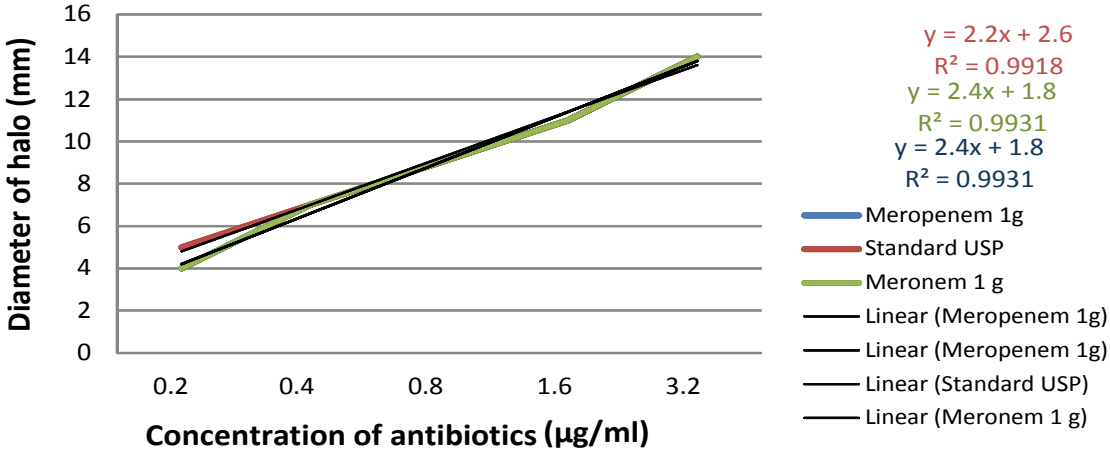

Figure 7. USP standard curve, meropenem and meronem with *Micrococcus luteus.*

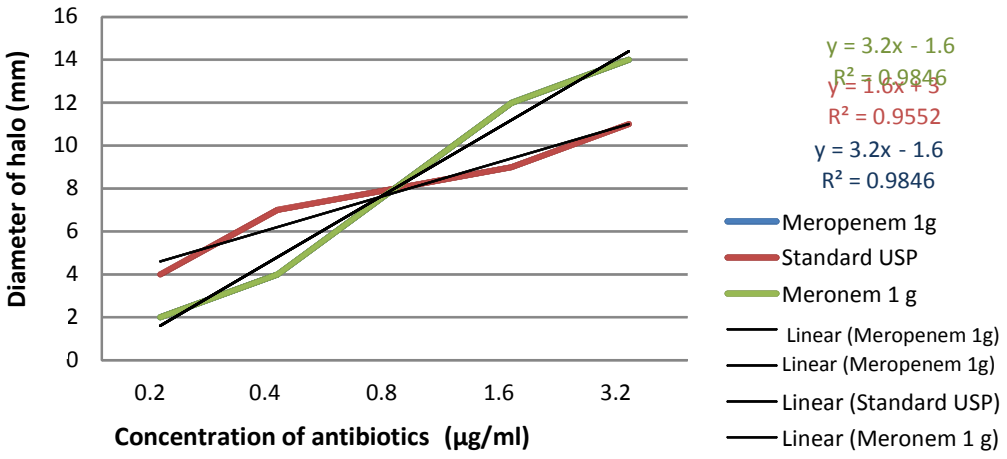

Figure 8. USP standar curve , meropenem and meronem with *S.aureus.*

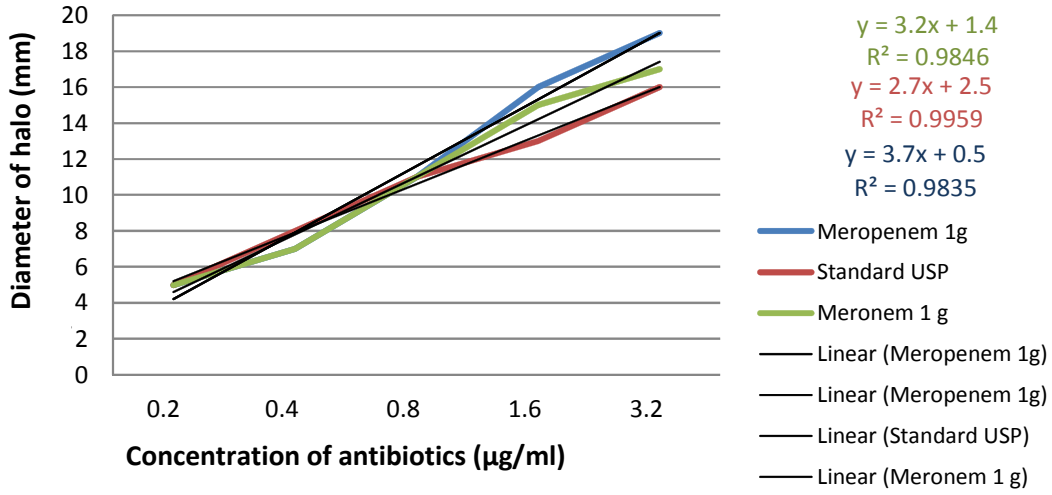

Figure 9. USP stándar curve, meropenem and meronem with *E.coli.*

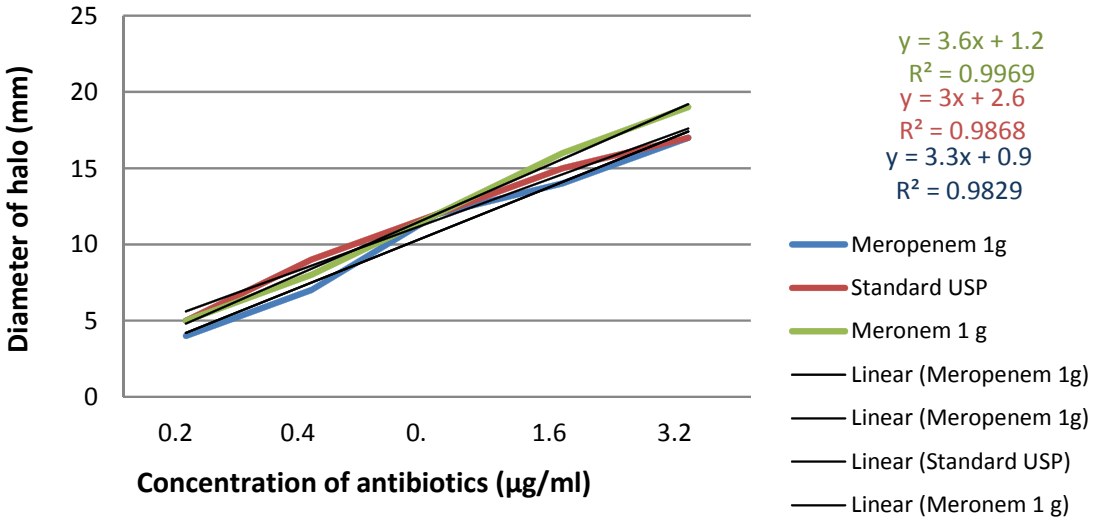

Figure 10. USP stándar curve, meropenem and meronem with *Pseudomona aeruginosa*.

DISCUSSION

The standardization of the antibiotic means that each of the microorganisms allows for a more accurate result of the inhibition zones, taking into account that each antibiotic medium (number 1 and number 11), only varies in pH, and thus it is possible to confirm that each evaluated microorganism holds varying conditions for its optimum growth. The r^2 obtained for each of the studied curves both of standards and the samples showed data of ≥0.98 which are placed within the acceptance range, showing that there is a relation between the values of both axis (Fernández et al., 2006).

Generic drugs generate much discussion about its competition with innovative medicines. The advantage of its use is its low cost, since it has no patent; in Colombia it is usual that health institutions and low-income users prefer generic drugs because they generate savings. The results of this research have great scientific and socio-economic impact, as it seeks to show that the generic drug is pharmaceutically equivalent to the innovator, demonstrating its quality and good manufacturing practices in their execution and therefore the same activity (Giner et al., 1995).

Taken together the data obtained by measuring halos in the standard curve is a reference on the feasibility of testing in statistical terms and this gives a criterion of analysis as valid ((Jones et al., 2004). By plotting the curves of the secondary standard VICAR, and meronem meropenem 1 g against microorganisms, *B. subtilis*, *M. luteus*, *S. aureus*, *E. coli* and *P. aeruginosa*, it is evident that the values are on the left side of the standard, which means that the declared capacity is accurate (Figures 1, 2, 3, 4 and 5). Taken together the data obtained by measuring halos in the standard curve is a reference on the feasibility of testing in statistical terms and this gives a criterion of analysis as valid.

According to the averages obtained from the potency percentage, there is no significant difference regarding the power of the different samples, since according to the USP XXXI (2008) for microbiological tests for all antibiotics, the percentage should be between 80 and 125%, since it is a test that has a lot of variables and possible sources of error. However, for the *Bacillus subtilis* microorganisms, the percentage of antibiotic potency of generic and innovative compared to the USP standard curve showed that there is a difference with the declared potency, because the values were 78.18% and 78.47% respectively. It is recommended that verifying the therapeutic equivalence to ascertain whether the generic antibiotic after administration of the molar dose, the effects on safety and efficacy are the same should be considered for further studies. In addition, understanding and manipulating various statistical programs and keeping concentrations antibiotics made for cooling and where light does not reach, for proper operation could also be ascertain.

Conclusion

This study verified the pharmaceutical equivalence of a generic meropenem vs. innovative by microbial power valuation. It was found that the coefficient of determination was equal to or greater than 0.98, determining the consistency of the results. Pharmaceutical equivalence was demonstrated for meropenem vs generic form through innovative technique plate cylinder. Pharmaceutical equivalence exists between the antibiotic and the innovative generic meronem meropenem for microorganisms *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*.

Abbreviations

WHO/PAHO, World Health Organisation/ Pan American Health Organisation; **FDA,** Food and Drug Administration; **r2,** coefficient of determination.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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