Review Article

An Analysis of *In-Vitro* Bioequivalence Studies and their Methods

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Received: 08-02-2023; **Revised:** 20-04-2023; **Accepted:** 26-04-2023; **Published on:** 15-05-2023.

ABSTRACT

The pharmaceutical business focuses a significant amount of attention on discovering an ideal alternative medication for an existing medicine while simultaneously preserving the bioavailability of the latter in the present day. A bioequivalence study is a type of research that is conducted with the purpose of comparing and contrasting the many various pharmaceutical formulations. The dissolving test is one of the simplest ways that may be used for determining whether or not two substances are bioequivalent to one another. This examination is also one of the most essential ones. The *in-vitro* bioequivalence and associated metrics, as well as previous research efforts in the field, will be the major focus of this analysis. This evaluation will also take into consideration the research that has been carried out in this industry. The study that has been done in this field will also be taken into consideration in this review that will be done.

Keywords: Biowaiver, Bioequivalence, *In-vitro*, Solubility, Compatibility, Fit-factor.

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DOI: 10.47583/ijpsrr.2023.v80i01.013

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v80i01.013

INTRODUCTION

The word "bioequivalence" is used to describe the practice of comparing and contrasting two or more pharmaceutical brands or dosage forms of the same drug. When the solubility and absorption rates of two medications are equal, we claim that they are bioequivalent to one another. Demand for bioequivalence studies rises in lockstep with the rate at which generic versions of existing drugs are being developed and implemented. The initial investment in the drug is what drives the subsequent increases in the cost of medications, and this tendency is projected to persist. Using generic versions, which are typically less expensive than their branded equivalents, is one way to save money here. This means that the therapeutic effects of the brand-name medicine and the generic equivalent should be same. Research on bioequivalence is being conducted to find out. According to the FDA, "bioequivalence" is defined as follows:

"The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study".

The two most common methods for conducting a bioequivalence study are in-vitro bioequivalence research and in-vivo bioequivalence studies. When a drug has been tested on humans or animals, an in-vivo bioequivalence research is commonly performed to ensure that it has the same effects. This study involves assessing the rate at which a drug is absorbed into the bloodstream as well as the total amount of drug that is absorbed. The findings of an in-vivo study are quite reliable, despite the fact that it is impossible to fully control a large number of the variables that were examined. In addition to this, there is a greater degree of diversity among living things. So, we need to carry out a lot of tests, and the associated costs must also be taken into account.

Research on the in-vitro bioequivalence of a substance is carried out using apparatus designed to make dissolution easier. In addition to the creation and upkeep of all of the necessary biological conditions for the experiment, standard procedures include the taking of samples and conducting analyses on them. By doing our research in-vitro, we are able to have a certain amount of control over the system. In addition to this, it enables the simulation of the conditions that are present in biological systems. It is possible that the costs associated with the trials will be greatly reduced owing to in-vitro research. In addition to these advantages, it also helps increase medication performance and addresses ethical concerns. A biowaiver is an exemption that is given by the Food and Drug Administration of the United States from the requirement to do in-vivo bioequivalence research (FDA). It creates the

idea that doing in-vivo research for the purpose of product approval is not necessary for generic copies of a medicine that are already on the market. Doing a dissolving test is another approach that might be considered. Items that are solid, orally administered, rapid-release, and contain highly soluble medicines across the pH range of 1 to 7.5 are eligible for the biowaiver scheme. While performing bioequivalence research for the purpose of obtaining a waiver, it is essential for both the test product and the reference product to have a dissolution profile that is equivalent to one another (f2 > 50). Nevertheless, it cannot be utilised in the production of buccal, sublingual, or oral dispersion formulations, nor can it be utilised in the production of modified release pharmaceuticals. The application of biowaiver helps reduce the amount of money necessary to bring breakthrough items to market. It is a substantial advantage that has the potential to cut down on the length of time necessary for the approval of a product.

ANALYSIS OF BIOEQUIVALENCE IN-VITRO:

Uniformity of content:

In order to perform our "bioequivalence" study in an efficient and effective manner, we must first understand whether or not there are changes in the percentage content of the active components. On a regular basis, you should measure the percentage of medication that is included in a tablet in order to assess whether or not the tablet has the appropriate dosage of the active ingredient. This may be done by dividing the total amount of medicine by the tablet's weight. The findings of an examination of the effectiveness of the treatment when it was administered in tablet form indicate to the presence of the drug in dosage form as well as to the consistency of the tablets. The test for content uniformity is outlined in the monographs for all dosage forms and random samples of tablets are selected and individually analysed for quality control. This is done so that every dose of medication in every pill is consistent. There should be no more than a 25% discrepancy between the test content and the advertised potency in tablets. A maximum of 15% variation in test content from the specified potency is permitted for tablets. If you want your dose units to stay the same size and shape after being compressed, you need to make sure they have a consistent weight[1].

Weight variation:

The weight of a tablet may be altered by a range of variables, including the compression machine's tooling, the head pressure, the machine's operating speed, and the powder's flow properties. Twenty tablets of each brand are used in the calculation to establish the amount of variation in weight that exists between the products. While measuring tablets, it is usual practise to use a weighing scale designed specifically for analytical purposes. We were able to establish the average weight for each brand as well as the % variation by using the mean value. This allowed us to compare the brands more accurately. There should not be more than two individual weights that are significantly different from the average weight, as suggested by the pharmacopoeia[1].

Hardness:

The tablet's resistance to chipping, abrasion, or breaking while being kept, transported, and handled prior to being put to use is measured by the hardness test, which is why it is so important. The overall weight of the material being used, the amount of pressure exerted during compression, and the amount of space left between the upper and lower punches all play a role in determining the ultimate hardness of the tablet. Many different kinds of testing equipment, such as the Monsanto or Stokes hardness tester, the Pfizer hardness tester, the Strong cob hardness tester, and the Heberlain or Schleeniger hardness tester, are used for the purpose of assessing the degree to which a material is abrasive.

Friability:

When a tablet's surface is damaged or shows a damaged region due to mechanical shock, this condition is known as friability. Friability can occur when a tablet is dropped or subjected to other types of mechanical stress. During this particular test, we keep an eye on how the tablet's edges behave in order to identify whether or not they come loose from the device. The friabilator manufactured by Roche was the piece of machinery that was put to use. It is essential to figure out the starting weight (W1) of 20 tablets that have been chosen at random. The tablets need to be run through the friabilator for a full four minutes at a speed of 25 revolutions per minute before the final weight (W2) can be determined. % The following is the computation that was utilised to calculate the total amount of loss:

$$\%\ Friability = \left(\frac{W1 - W2}{W1} * 100\right)$$

Disintegration:

Disintegration research is necessary for estimating the rate and extent of medication release. It is possible to assess how long it takes for tablets or capsules to completely disintegrate into their component components by performing a test that is referred to as a disintegration test. In order to assess whether or not there was any consistency in the compression characteristics, a first test called a disintegration test was carried out. This test is the one that we favour using these days in order to get the best possible results for optimising compression quality. A pill that takes an abnormally lengthy time to dissolve may be heavily compressed or made with a lower-quality gelatine capsule shell. In the event that the disintegration time does not remain constant across batches, this will result in a lack of batch homogeneity in addition to inconsistency among batches. Although the particulars of the disintegration apparatus change depending on the kind of drug that is being digested, the idea that underpins them and the method that they are constructed remain the same. The

apparatus consists of a basket that is comprised of six tubes that are all the same diameter as one another. A piece of metal mesh has been soldered onto each and every one of these tubes. A motor that turns in a circle is responsible for helping to move the basket in the desired direction. The whole unit is maintained in a container that is submerged in the medium under test for the duration of the test.

Dissolution test:

The efficacy of the dose is determined by the amount of medicine that dissolves in the bodily fluids as well as how well it is absorbed into the systemic circulation. As a result, it is essential to do a calculation to determine the rate of dissolution of a dosage form. In a dissolving apparatus, the maintenance of the necessary biological conditions is accomplished by supplying the proper dissolution media and controlling the temperature using a thermostat. At certain intervals, samples are taken for analysis. In order to preserve the conditions of the sink, an equal quantity of media is continually introduced. In accordance with this, the tests are carried out. The choice of dissolving medium, apparatus, and agitation rate all play a vital part in producing accurate results from a dissolution test. The dissolution test has been carried out for,

- Throughout the product development and stability testing process, the therapeutic efficacy should be optimised.
- Evaluation of the product's quality on a regular basis to guarantee consistency across different production batches.
- Evaluation of the "bioequivalence" of two substances.
- Prediction of the availability in living organisms, often known as bioavailability (where applicable).

In accordance with the Pharmacopeia, several kinds of equipment are utilised for this purpose depending on the dosage form being evaluated. The following is a list of them as per regulatory guidelines[2,3].

Dissolution Apparatus and Details as Per USP		
Apparatus type	**Name**	**Drug product type**
Apparatus – I	Basket	Tablets, capsules
Apparatus – II	Paddle	Tablets, capsules
Apparatus – III	Reciprocating cylinder	Modified tablets, capsules & beads
Apparatus – IV	Flow-through cell	Low soluble active ingredients
Apparatus – V	Paddle over disk	Transdermal dosage forms
Apparatus – VI	Rotating cylinder	Transdermal dosage forms
Apparatus – VII	Reciprocating holder	Extended-release dosage forms

ANALYTICAL PARAMETERS:

The amount of medicine that has dissolved should be more than eighty percent of the amount that is indicated on the label during the first thirty minutes after taking the drug. The constraints imposed by the Pharmacopoeia necessitate that this be done. After 15 minutes, if the drug has dissolved at a rate of more than 85 percent, further mathematical analysis is not required. Mathematical evaluations were carried out in order to demonstrate that bioequivalence might be accomplished with the use of chemicals that did not fulfil the standards. Calculations such as the fit factor Dissolution efficiency, Correlation-Coefficient, ANOVA test, and Dunnett's test are all part of the quantitative analysis. Fit factor is also known as the Similarity and dissimilarity factors. The fit criterion is sometimes referred to as the similarity and dissimilarity indices.

Fit factors

The fit factors can be expressed by two approaches: Similarity factor (f2) and

Dissimilarity factor (f1).

Similarity factor (f_2):

When it comes to making comparisons of in-vitro dissolution profiles, the Food and Drug Administration of the United States places a significant amount of weight on the criterion of similarity and difference. The comparison of how closely two similar formulations is connected to one another is the primary focus of the similarity factor (f2), which, as its name implies, lays an emphasis on the topic. In the process of establishing whether or not two different dissolution profiles are comparable to one another, the f2 parameter is utilised rather frequently as an aiding tool. The Food and Drug Administration (FDA) describes the similarity factor as "the logarithmic reciprocal square root transformation of one plus the mean squared (the average sum of squares) differences of drug percent dissolved between the test and the reference products." This definition can be found in the FDA's official glossary. The similarity factor is determined by calculating the mean squared differences, often known as the average sum of squares. The formula that may be applied in order to calculate the degree of similarity between two things is as follows:

$$f2 = 50 + \log\{[1 + (\frac{1}{n})\sum t = 1 * n\,(Rt - Tt)^2]^{-0.5} \times 100\}$$

Rt and Tt are abbreviations that stand for the cumulative percentage of the reference product that has dissolved and the percentage of the test product that has dissolved, respectively, at each of the n time intervals that were chosen. At the point where f2 = 100, two profiles are compared and found to be identical to one another. The value of f2 is determined to be 50, with a variance of 10% on average across all of the time points that were measured. If the value of f2 is between 50 and 100, this

indicates that the dissolving profiles of two different substances are comparable to one another. To put it another way, the value of f2 will be within the range of 50 to 100 if and only if the difference between each sample time is less than or equal to 10%. This is the only condition under which this range will be satisfied. In light of this, a speedy method for determining whether or not two profiles are comparable is to check whether or not the variations in dissolution findings at each sample period are less than 10%. This can be done by checking whether or not the variations in dissolution findings at each sample period are less than 10%. There is a possibility that the limit of the 50 to 100 range for f2 is in conflict with the criteria established by the pharmacopoeia that is used the most, such as USP. These criteria state that the allowable deviation must be significantly greater than 10%. There is a chance that this conflict exists. For analyzing things that have a formulation and assessing whether or not there have been any modifications in the production process, it is advisable to utilize the f2 function. When there are two groups that are equivalent to one another, namely the reference group and the test group, f2 will equal 100. As the degree of dissimilarity increases, f2 becomes closer and closer to zero, until it ultimately gets there.

Dissimilarity factor (f_1):

The dissimilarity factor compares the reference value to the test value at each of the distinct time periods and determines how big of a difference there is between the two values. As a consequence of this, the variables conduct an indirect comparison of the variance between a test product and a reference product about the percentage of medication that dissolved in a given amount of time. Using the f_1 factor is necessary in order to calculate an approximation of the proportion of error that exists within a pharmaceutical release profile. f1 has in between 0 to 15 for proper functioning. The following is the formula for the dissimilarity factor, often known as f_1:

$$f_1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100$$

The United States Food and Drug Administration lists the following factors as those that are relevant to the dissolution profile:

1. A comparison of dissolution profiles is only possible when using a total of at least 12 dissolution units. The mean dissolution rate from 12 individual doses should be used to calculate f2.
2. It is possible to employ a statistical method known as the development of confidence intervals in order to arrive at an accurate computation of the similarity factor. This method is used to assess whether or not the reference and test are statistically significant.
3. The parameters of dissolving should be the same for both the reference product and the test product. These conditions include the strength of the dosage form, the test time intervals, temperature, rpm, and total test time.
4. According to the research, there should be only one time point considered after 85% of the product has dissolved since f2 values are sensitive to the number of different dissolving time points.
5. Comparison of dissolution profiles is not required for goods that have a quick dissolving time, such as those that can dissolve 85 percent in 15 minutes.
6. A similarity value between 50 and 100 indicates that two goods are identical.
7. A difference factor in the range of 0 to 15 will ensure that there is only a subtle distinction between the two items.

Comparing in-vitro dissolution profiles with the help of fit factors is thus likely to be the most promising surrogate prior to doing studies in animals. Although computing the Similarity factor in order to compare dissolution profiles is an important step, the issue of variability in dissolution data was not taken into consideration by this step. These technologies are also helpful in the process of formulating new products.

Dissolution efficiency:

The term “dissolution efficiency” refers to the proportion of the area under the dissolution curve that corresponds to maximum dissolution Y100 that occurs during a certain time interval (t1 - t2).

$$DE = \frac{\int_0^T dt}{Y100 \times T} \times 100\%$$

Studies of bioequivalence performed in-vitro are the subject of the following scholarly articles:

Tanjinatus et al[4] study's used ten different generic brands of Atorvastatin, each containing 10 milligrammes and designated by a letter from A to J, for their “in-vitro bioequivalence investigation. The diffusion of drugs in both the A and D brands was studied in-vitro. Atorvastatin was dissolved in both methanol and water for this study, and both were evaluated for efficacy. The weight consistency tests failed both brand C and brand E. Not only was there noticeable variation in dissolving profiles between brands, but also between batches of the same brand. Dissolution rates in-vitro were shown to be correlated with membrane permeability, which was investigated by methods of in-vitro diffusion. Brand D, with a much higher in-vitro disintegration rate, was able to easily pass through the membrane. A comparison to Brand D using the fit factor analysis showed that any of the brands may serve as a suitable replacement. Brand D worked quite well as a solvent. Brands B, C, I, and H, all have roughly the same dissolution efficiency as Brand D and can be used interchangeably.

In order to identify which of the several brands of chloroquine phosphate syrup sold in Nigeria are similar, Tense and Ibrahim performed in-vitro bioequivalence testing[5]. The study compared five different brands of immediate-release chloroquine phosphate syrup. This report includes the results of three investigations. Chloroquine phosphate concentrations were all over the place. It was demonstrated that a profile of % absorbed versus time is more informative than either a concentration against time profile or an absorption volume against time profile. Absorption kinetics were found to be first order for Brands 1 and 2, but zero order for Brands 3 and 4. There was no evidence that Product 5 followed first- or second-order kinetics. It's been pointed out that there's no method to demonstrate bioequivalence across the five different chloroquine phosphate syrup brands. From the point of view of drug regulatory agencies, this conclusion emphasizes the significance of bioequivalence studies comparing different brands of the same medicine.

Eight different brands of the diabetic medication Metformin were tested by Parvin and colleagues[6]. Their bioequivalence was determined after a battery of tests. The average weight, standard deviation, and relative standard deviation of each brand were determined. All drugs were confirmed to be within the 15% tolerance limit, and dosage consistency was obtained. Usual Pharmaceutical Practice (USP) rules dictated the usage of paddle-style dissolving equipment. The 900 mL of phosphate buffer used as the medium. Hundredfold dilutions were made of the materials before being examined under UV light. The concentration may be calculated thanks to the calibration curve. Research on the in-vitro bioequivalence of ciprofloxacin was reported by Ngwuluka and colleagues, who found that 80% of the drug was released after 30 minutes across all brands except Vitapro, which had 77% at 35 minutes[7]. In order to conduct the experiment, six different brands of Ciprofloxacin, each containing 500 milligrams, were procured in Nigeria. Powdered ciprofloxacin HCl was chosen as the gold standard by the group. Cifrofloxacin's bioequivalence has been the subject of much research. To my dismay, however, brands C, D, and F did not make the BP cut. The rigorous standards set by the USP were satisfied by all of the available brands. Uncoated BP products have an estimated dissolution time of 15 minutes, while film-coated products require 30 minutes. Both uncoated and film must be entirely deteriorated within 30 minutes to meet USP criteria. All the brands except B and F (A, C, D, and E) were uncoated. We measured the rate of disintegration using the USP basket test. B had distributed 92% of the medication by the 15-minute mark. In most cases, rival manufacturers provide less than 85% of the whole quantity. The eliminated percentage was determined using Dunnett's test and one-way analysis of variance (ANOVA). Element B was the basis for comparisons to A, C, D, and E in a pairwise analysis. Dissolution effectiveness tests were also conducted for good measure. A and E were found to have comparable solubility profiles, which is indicative of their bioequivalence to B. Because of this, you may use any one interchangeably.

An examination of the in-vitro bioequivalence of Sulfisoxazole tablets was conducted by Pandey and Pandit[8], and their findings were reported here. The Sulfisoxazole tablet was formulated into three different forms: two chewable (types C1 and C2), two swallowable (types S1 and S2), and one commercially available (type C3). Disintegration and friability tests, as well as solubility, hardness, weight variation, and friability tests, were carried out. Despite S1's decreased hardness, integration durations were substantially comparable to those of the reference, demonstrating that hardness was not the determining factor. An S2 sample with a little greater hardness took noticeably longer to disintegrate. S2 disintegrated almost as rapidly and thoroughly as a conventional tablet. Time to disintegrate for S1 was much faster than that of S2.

Ramu and Srinivasa Babu authored and published an article evaluating the effectiveness of Glimepride tablets in-vitro[9]. Two milligrammes of each branded Glimepride preparation (GM1–GM4) and one milligramme of pure Glimepride were used in this study. Many experiments were conducted, some of which included changing the weight of the sample while others tested its hardness, friability, disintegration, and solubility. The dissolving test was conducted in a paddle-type apparatus at a rotational speed of fifty revolutions per minute. In this study, we utilised 0.1NHCl, a phosphate buffer at pH 7.4, and 0.5% sodium lauryl sulphate in distilled water as dissolving medium. The degree of friability was always found to be under 1%. Greater than other GM2 release rates (order: GM2, GM3, GM4, GM1). The medication was released more quickly in the medium containing 0.5% SLS compared to pure water. Branded glimepride considerably outperformed pure glimepride in every dissolution metric.

Alcohol's impact on extended-release medications was the subject of a recent paper by Anagha Joshi and colleagues[10]. Both metformin and diclofenac were studied to see if their release was affected by alcohol use. This experiment used four types of alcohol: a 500 mL bottle of Kingfisher strong, a 500 mL bottle of Kingfisher light, a 60 mL bottle of rum, and 40% alcohol (15 mL). The various drugs' dissolvability was tested. There was a positive correlation between the concentration of alcohol and the rate of metformin release, but no correlation between the two. The sustained-release version of diclofenac was found to have a release profile that was quite similar to the immediate-release form. Instant release formulation was more rapidly absorbed by the body when mixed with Kingfisher strong beer, mild beer, Rum, and 40% alcohol compared to water. Researchers found that the sustained-release formulation worked better in rum and 40% alcohol than in water. Researchers observed that drinking alcohol while on therapy with sustained release formulations altered the drug's dissolving profile and contributed to the emergence

of a number of unintended consequences, including dosage dumping.

Ashraful Islam and colleagues evaluated the bioequivalence of Aceclofenac tablets in two dissolving media and published their findings[11]. Five different brands of 100 milligrammes of Aceclofenac were created to test its solubility. UV spectroscopy and high-performance liquid chromatography were used in their investigation. Results from the tests were used to determine concentration by examining the peak area, which is indicative of maximal activity. Twenty pills were extracted, counted, crushed, and dissolved. To complete the dissolving, 100 mL of phosphate buffer with a pH of 6.8 is used. After being shook for 10 minutes, this was sonicated for five. Dilutions were made until the final volume was 100 mL and the concentration was 12 mcg/mL. When the solution had been sonicated, it was put through a dissolving test using a paddle to see if the particles were able to dissolve. The dissolution was carried out in a solution of phosphate buffer and sodium lauryl sulphate. High-performance liquid chromatography (HPLC) produces reliable results because it is very selective and produces a peak of the same size for both the standard and the sample. It was determined that 900 millilitres of pH 6.8 phosphate buffer, 37 degrees Celsius (plus or minus 0.5 degrees), and a paddle speed of fifty revolutions per minute produced the best results when used to synthesise aceclofenac.

Nifedipine is a vital medication for treating cardiac issues. Panchagnula and colleagues conducted a bioequivalence study comparing nine different nifedipine brands sold in India[12]. They were assigned the numbers N1 through N9. Several types of tests, such as those for weight variation, friability, dissolution, assay, and hardness, are carried out. We employed a dissolving apparatus resembling paddles for our dissolve tests. A 6.8-pH phosphate buffer with 1% SLS was used. The impact of a pH shift from 2.0 to 5.0 and then to 7.4 was also studied. The NIPER formulation's dissolving profile was compared to a reference profile based on fit-factor analysis. Studies of dissolving N1 indicated that the medication release process began after two hours. Although N3, N4, N5, N7, and N9 released the drug slowly, N6 and N8 released over 80% of the drug in about one hour. Only N1 and N7 were examined further beyond the initial two. The experimental results indicated that N1 dissolved with both zero and first order kinetics, while N7 dissolved only with first order kinetics. Both N1 and NIPER were rated more effective than the market's existing selection of pharmaceuticals.

In Lagos State, Nigeria, Olubukola et al. investigated the in-vitro equivalency of generic Metformin hydrochloride tablets and Propranolol hydrochloride tablets under biowaiver circumstances[13]. All the drugs included as illustrations were the standard, immediate-release, oral solid-dosage kind. Glucophage (glucophage xr) and Inderal (propranolol hydrochloride 40 mg tablets) are two examples of commonly prescribed medications (brand name for metformin hydrochloride 500 mg tablets). Two generic 40 milligramme propranolol hydrochloride tablets and four generic 500 milligramme metformin hydrochloride pills were utilised in the study. In order to establish individuality, both propranolol and metformin hydrochloride were used. Both generic and brand-name options were evaluated according to BP requirements for their active components. Several liquids were used in the solubility tests: Phosphate buffer (pH 4.5) in a ratio of 2 to 1, with more phosphate buffer, and 0.01 N HCl acid (pH 2). (pH 6.8). All of the results from the tests done on the propranolol hydrochloride tablet samples were within the parameters set out by BP. Both generic and name-brand propranolol hydrochloride tablets decomposed fast, releasing over 85% of the indicated quantity within 30 minutes. After 15 minutes, the dissolution rate for only one of the four metformin brands examined was 85% or greater. But, after just 15 minutes, the generic form disappeared from all three channels by more than 85%. In this study, the branded form of propranolol hydrochloride was compared to two generic alternatives. The similarity factor (f2) was used to evaluate the correspondence between the dissolution profiles. According to the calculated f2 values, there was insufficient evidence to conclude that any of the generic samples were statistically indistinguishable from the gold standard. It was concluded that none of the generic medications examined were eligible for a biowaiver. In-vitro dissolution studies to determine bioequivalence are required for regulatory submissions. Manufacturers might take into account aspects like solubility and permeability in the creation of their goods.

Recent research by Patrico JP et al. evaluated the in-vitro solubility patterns of two widely available iron solutions. Two commercially available iron and folic acid supplements were compared in an in-vitro dissolution research. Brands like Folifer® (made by Bialport - Produtos Farmacêuticos, S.A., Portugal) and Ferroliver® are available". Portugal is the source of these two formulas. They were chosen because their iron content was almost identical. Three distinct dissolving mediums were employed, all of which were designed to simulate stomach acid. The pH scale has a wide range, from 1.5 to 6.9. The quantity of iron absorbed by the body from each tablet was measured over the course of a four-hour test. Titrations were performed on samples that had been treated with cerium ammonium sulphate to determine the release rate of iron over a range of pH conditions. Calculating the iron dissolution rate using a cumulative frequency. The dissolution similarity was analysed with the 2-statistic method. Folifer® outperforms Ferroliver in terms of iron liberation, the study found; the two are not comparable.

Many brands of Levofloxacin pills (250 mg) are now available on the black market in Karachi (Pakistan); thus, Raheela ban et al. undertook study to evaluate their pharmaceutical quality[14]. In this study, we used levofloxacin from six distinct manufacturers (Levo 1 through Levo 6). Each of the six tablets tested conformed to both USP and BP specifications. The thickness of Levo 6

was found to be much higher than that of any of the other tablets tested. Chemical examination of levofloxacin pills showed that the actual amount of the active component was between 95% and 105% of the labelled amount. Analysis of variance (ANOVA) results indicated no statistically significant variation in total active moiety concentration. The results of this study made it abundantly evident that the difference in pill weight and thickness has a substantial effect on patients' propensity to take their medication as prescribed. Also, they no longer have any significance when considered alongside the fact that the tablet thickness has been adjusted accordingly. Amounts of levofloxacin in different brands ranged from 95% to 105%, therefore it can be concluded that the concentrations of the active component in each brand were within the range specified by the pharmacopoeia.

Chandrasekaran and coworkers wrote and published a paper on the in-vitro bioequivalence evaluation of paracetamol tablets[15]. In this study, researchers used six branded 500mg paracetamol tablets. Weight, hardness, friability, and disintegration time variations were kept within the ranges allowed by USP and BP. The USP apparatus I was used to conduct the dissolution test, and a phosphate buffer at pH 5.8 was used as the dissolving media. Customers may conveniently obtain products from brands B, C, D, E, and F. It appears that all six varieties examined dissolved similarly, and there was no noticeable variation between the different manufacturers.

Ochekpe and coworkers tested the bioequivalence of 12 brands of Sulphadoxine pyrimethamine available in Nigeria[16]. For the experiment, the scientists utilised three unique solvents. Solubilizing conditions included 0.1 N HCl and phosphate buffers at pH 4.5 and 6.8, respectively. In this specific study, disintegration test equipment II was used. The United States Pharmacopeia (USP) recommends a phosphate buffer with a pH of 6.8 for a medication release in the medium of greater than 60% after 30 minutes. Brands 2, 4, 5, 7, 8, 9, and 10 had higher than 60% of both active components released after 60 minutes, whereas Brand 1 had less than 50% and Brand 11 had less than 20%. Brands 2, 5, 7, 8, and 10 were shown to be statistically equivalent to the gold standard in f1 and f2 analyses.

Balaji and Sultana reported on the genotoxic impurities in Febuxostat drug substance and products[17]. In this study researcher found that four possible genotoxic impurities in febuxostat have been identified using this approach. Elution is carried out in a linear gradient using a mobile phase composed of trifluoroacetic acid, acetonitrile, and water. A 100 mm long, 2.1 mm ID, 1.8 m particle size Zorbax RRHD eclipse plus C18 UHPLC column was employed for the study. Impurities may be detected at a detection limit of 0.1 (0.00001%) and quantified to a limit of 0.3 (0.00003%) g/mL relative to a test concentration of 1000 g/mL febuxostat. The ICH Q2 requirements have been satisfied in validating this procedure (R1). The quantitative analysis of potential genotoxic contaminants in febuxostat drug substance and drug products can now be performed with great success because to the excellently established quick, cost-effective infinite LC technique.

Muchakayala SK et al[18] developed an accurate, simple, and rapid UPLC method for the determination of impurities present in cream and ointment formulations of Betamethasone dipropionate. Optimized chromatographic separation was achieved using a Waters Acquity UPLC BEH C18, 100 mm × 2.1 mm, 1.7 µm column with a gradient mode of elution comprising 20 mM phosphate buffer: ACN 70 : 30, v/v as mobile phase-A and 20 mM phosphate buffer: ACN 30 : 70, v/v as mobile phase-B. The developed method was validated in accordance with ICH guidelines. Balaji and Sultana published a paper on the determination and quantification of potential genotoxic impurities in Dasatinib drug substance[19]. Three potential genotoxic contaminants in Dasatinib were measured using this technique. Trifluoroacetic acid, acetonitrile, and water make up the mobile phase, and elution curve 6 is a linear gradient. In both cases, we relied on a Zorbax RRHD Eclipse Plus C18 column that measured 50 mm in length, 2.1 mm in internal diameter, and 1.8 m in particle size for our testing and development. When measured against a Dasatinib test concentration of 1000 ng/mL, the lowest detectable levels of potentially genotoxic contaminants are less than 0.1 ng/mL. In tests with a Dasatinib concentration of 1000 ng/mL, the detection limit for possible genotoxic contaminants is less than 0.3 ng/mL. The ICH Q2 guidelines were used to verify this procedure (R1). The only process-related impurities found in Dasatinib are these three possible mutagens. The QbD concept has guided the method's evolution.

Srinivas et al[20] wrote and published a paper on the “Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of Tolperisone HCL and Etodolac in bulk and its pharmaceutical formulation”. RP-HPLC was used to estimate Tolperisone and Etodolac1,2,3 in pure and tablet forms. The procedure was ICH-validated35. A mobile phase of potassium phosphate monohydrate buffer (pH-2.6) and acetonitrile (70:30% v/v) was utilised using a C18 column (250×4.6mm, 5µm). Run duration was 6 minutes at 1mL/min. Tolperisone and Etodolac were eluted at 2.8 and 4.2 min, respectively, at 263nm. The approach was linear (r2 =0.999) at concentrations of 7.5 to 25µg/mL for Tolperisone and 100-300µg/mL for Etodolac, exact (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (99.3 to 100.9 for Tolperisone and 100.1 to 100.6 for Etodolac), specific, and robust. Tolperisone and Etodolac detection limits were 1.30 and 1.88 µg/mL. Tolperisone and Etodolac quantitation limits were 3.93 and 5.70 µg/mL, respectively. The findings demonstrated that the suggested approach can accurately, quickly, and precisely determine bulk Tolperisone and Etodolac dose forms.

Srinivas et al[21] worked on the “Method development and validation of stability indicating RP-HPLC method for

simultaneous estimation of Atazanavir and Ritonavir in bulk and its pharmaceutical formulations". Using a C18 Phenomenex column (250 mm 4.6 mm, 5 m) and a mobile phase of 900 mL of HPLC grade methanol and 100 mL of HPLC grade water, a stability-indicating RP-HPLC technique was designed and validated for the measurement of atazanavir sulphate in tablet dosage forms. With acetic acid, the pH was lowered to 3.55. At a flow rate of 0.5 mL/min, the mobile phase was sonicated for 10 minutes before being filtered through a 0.45 m membrane filter. Atazanavir sulphate has a retention duration of 8.323 minutes when measured with a 249-nm detector. The concentration range tested was found to be linear (R2 = 0.99) with the following equation: y = 23.427_{χ}+37.732. Results indicated that Atazanavir sulphate was more susceptible to acidic degradation when tested under stress conditions such acidic, alkaline, oxidative, photolytic, and thermal degradation. Method validation was performed in accordance with ICH standards.

CONCLUSION

In the current industrial practise, in-vitro bioequivalence serves a significant purpose both to compare with the multi brand generic molecules and to supply sufficient therapeutic activity of the dosage form. This is the case because in-vitro bioequivalence may be performed in a laboratory setting. Because of both of these considerations, this action is taken. Our review paper compiles data on in-vitro bioequivalence studies, discussing their utility and the various methods used in bioequivalence analysis. This review article aims to provide a comprehensive overview of in-vitro bioequivalence studies, discussing their importance and the many methods used in this field. In addition, we have discussed the various methods that are used in bioequivalence studies. This information can be utilised for institutional as well as industrial practise in accordance with the restrictions and prerequisites outlined in the Pharmacopeia. The results of the post-marketing in-vitro bioequivalence tests conducted on many brands provide essential information on the relative levels of quality possessed by each of the brands. The conclusion is reinforced by the findings that were published by a number of writers utilizing a range of generic molecules and contrasting them with a variety of brand names. These data were used to draw comparisons between the generic molecules and the brands. The evidence that has been provided leads us to believe that in-vitro bioequivalence studies are not only necessary but also have the ability to affect dosage form manufacturing companies.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.