A REVIEW ON BIOEQUIVALENCE STUDY: IN VITRO-IN VIVO CORRELATION

Naman[1], Mansi Garg[1], Shweta Mishra[1*]

SGT College of Pharmacy,
Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, India 122505

Abstract

Bioequivalence studies play an important role in the pharmaceutical industry for the development of a pharmacological formulation. The monitoring of pharmacokinetic and pharmacodynamic variables following the administration of tested medications serves as their justification. The goal of such research is to assess the therapeutic compatibility of tested medications (pharmaceutical equivalents or pharmacological alternatives). The importance of bioequivalence studies is growing as the manufacture and usage of generic products grows. Together with the pharmaceutical quality data of the medical product, the search results of the bioequivalence research make up one of the major components of the registration file submitted to a national regulatory authority. It is recommended that a bioequivalence research be used to compare the original and the generic product is sufficient. The objective of present review article is to inform the medical community about the different kinds of bioequivalence studies, their application, and guidelines for their use, and to help them form their own opinions about the matter.

Keywords: In vitro-In vivo Correlation, Bioequivalence Study, pharmaceutical products, USFDA

[1*,]SGT College of Pharmacy, SGT University, Gurugram, Haryana, India 122505

***Corresponding Author:** Shweta Mishra
*****SGT College of Pharmacy, SGT University, Gurugram, Haryana, India 122505

DOI: - 10.48047/ecb/2023.12.si5a.0476

1. Introduction

Over the past two decades, the cost of healthcare has increased globally, which has led to initiatives in the majority of nations to lower those costs. It is well recognised that medication is used in the majority of healthcare procedures. The contribution of drug expenses to the overall expenditures of healthcare has drawn a lot of attention because the cost of medication has also been rising over time[1]. The introduction of generic versions of brand-name pharmaceuticals (also known as innovator drugs) into international markets has been a key approach for lowering the price of medication and, consequently, reducing its contribution to overall healthcare expenses. The tactic has worked well. From 1997 to 2000, the average national savings from using generic medications was around $9 billion, or 11% of all prescription prices. Due to its use with both new brand-name medications and generic medications during the past three decades, the concepts of bioavailability (BA) and bioequivalence have grown in significance. During this time, regulatory agencies also began to develop and formulate the legal specifications for the acceptance of generic drug products. The application of evaluation methodologies to these scientific notions has so made significant progress. Brand-name and generic pharmaceuticals are now approved on a global scale based on BA and BE, which are also used for brand-name drugs to lower development costs. The examination of several brands and dosage forms of the same medicine is known as bioequivalence. When the rates of dissolution and absorption are the same for two medication formulations, they are said to be bioequivalent. (1,2) The demand for bioequivalence studies is growing along with the manufacturing and usage of generic medications. Because original medications are so expensive, drug prices are rising today. You can cut this expense by using less expensive generic replicas. This requires that the generic version of the medicine be therapeutically equal to the original. Bioequivalent investigations are carried out to discover this. Bioequivalence is defined by the Food and Drug Administration as follows: It is defined as "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" (source). *In-vitro* and *in-vivo* bioequivalence studies are the two methods that are most frequently used to conduct a bioequivalence study[2]. It is common practice to measure the pace and degree of medication absorption in the blood stream after a medicine has been delivered in human and animal subjects for *in-vivo* bioequivalence studies. Although information from *in-vivo* studies is extremely reliable, there are many variables that are out of our control. Additionally, living things are more variable. Therefore, we must undergo a number of trials, and expense is important. The dissolve apparatus is used to conduct an *in-vitro* bioequivalence research. Samples are periodically collected and analyzed under all the required biological conditions. We are able to have control over the system by doing *in-vitro* research. It also enables the imitation of biological circumstances. Studies conducted in vitro cut down on trial costs and numbers. Additionally, it has advantages from an ethical standpoint[3].

2. General Considerations for Bioequivalence Studies according to FDA[4]

1. Study design and protocol
2. Bioanalytical methods and validation
3. Selection of appropriate analyte(s)—parent drug and/ or metabolite, prodrug
4. Bioequivalence metrics
5. Data transformation
6. Statistical approaches and analysis
7. Establishment of bioequivalence criteria

3. Biowaiver

To perform *in-vivo* bioequivalence research, the US FDA has given an exemption known as a "biowaiver." It implies that *in-vivo* studies are not required for generic products to obtain product approval. An alternative is to use the dissolution test. Only solid, oral immediate-release formulations that include highly soluble medicines over a pH range of 1 to 7.5 can be recommended for biowaiver (85% release in 30 min). The test and reference products should have a similar dissolving profile ($f2 > 50$) for a bioequivalence analysis of a waiver. However, it does not apply to formulations that are buccal, sublingual, oral dispersion, and modified release. The cost of introducing new items to the market is decreased via Biowaiver. It offers the significant benefit of speeding up the clearance process for a product[5].

The following are some benefits of employing biowaiver

- They avoid costly and occasionally dubious human testing.
- Shortens the time it takes to launch a product.
- Brings down product costs.

Regulatory organisations allow three different forms of biowaivers -

1.Biowaivers based on the BCS (Bio pharmaceutics Classification System)
2.Applications with a Long History or Bibliographic References
3. Submissions based on literature

4. Tests conducted to study *in-vitro* bio equivalence

4.1 Uniformity of content

To conduct a bioequivalence study, it is crucial to determine whether the percentage content of the active components varies. Drug content should always be assessed in percentages in order to verify that a tablet contains the right amount of medication.

Analysing the drug potency in tablets reveals that the substance is present in dose form and is stable. All dosage form monographs include the content uniformity test, and samples of tablets are chosen and assessed separately. Maximum tablets must have assay content that is between 15% and 25% of the advertised potency. When dose units are compressed, consistency is ensured by weight uniformity[6].

4.2 Weight variation

Tooling of the compression machine, head pressure, machine speed, and powder flow characteristics are some of the variables that determine tablet weight. Twenty tablets from each brand are taken in order to calculate weight variation.

The tablets are typically weighed using an analytical weighing balance. Average weights for each brand and % deviation were determined from the mean value. Pharmacopoeia states that deviations from the average weight should not exceed two individual weights[7].

4.3 Hardness

The hardness test is crucial because it establishes the tablet's resistance to chipping, abrasion, or breaking during handling, storage, and transportation before use.

The distance between the upper and lower punches at the moment of compression, the material's weight, and the pressure employed during compression all have an impact on the tablet's hardness. There are several different types of equipment used to measure hardness, including the Strong Cob Hardness Tester, Heberlain or Schleeniger Hardness Tester, Monsanto or Stokes Hardness Tester, and Pfizer Hardness Tester[8].

4.4 Friability

Friability is a phenomena in which a tablet's surface gets harmed or manifests a damage site as a result of mechanical shock. This test is run to ensure that the tablet's edges won't separate. The device in use is a Roche friabilator. The initial weight (W1) of 20 pills is determined at random. The final weight (W2) is computed following the tablets' 4 min at 25 rpm exposure to the friabilator. The formula is used to calculate the percent loss[9].

$$\% \text{ Friability} = ((W1-W2)/W1) * 100$$

4.5 Disintegration

Disintegration research is crucial for assessing medication release. Disintegration tests are conducted to determine the length of time it takes for the tablets or capsules to completely dissolve. Disintegration testing was previously used to determine the consistency of compression characteristics. These days, we favour this test for improving compression qualities.

If the disintegration time is excessive, the pill is tightly packed, or the gelatine in the capsule shell is not of the requisite calibre. Disintegration times that vary lead to inconsistent batches and a lack of batch uniformity. For various medications, there are many types of disintegration apparatus, but the basic design and construction are the same. The tool is a basket with six tubes within that are all the same diameter. Each of these tubes has a wire mesh attached to it. The basket is moved by a reciprocating engine. The complete assembly is kept submerged in a container containing the testing medium[10].

4.6 Dissolution test

The amount of medicine dissolving in body fluids and its absorption into the systemic circulation determine the dosage effectiveness. Therefore, it's crucial to determine how quickly a dosage form dissolves.

A thermostat-controlled dissolution equipment maintains biological conditions by supplying the proper dissolution media and temperature. At regular intervals, samples are taken out. An equal amount of media is supplied in order to maintain sink conditions. Assays are conducted as necessary. The choice of dissolve medium, apparatus, and agitation rate are crucial for a successful dissolution test[11].

Table 1 : Dissolution Apparatus and Detail as per USP

DISSOLUTION APPARATUS AND DETAIL AS PER USP		
APPARATUS	**NAME**	**DRUG PRODUCT**
Apparatus I	Rotating basket	Tablets
Apparatus II	Paddle	Tablets. capsules modified drug products
Apparatus III	Reciprocating cylinder	Extended-release drug products.
Apparatus IV	Flow cell	Drug products containing low-water-soluble drug
Apparatus V	Paddle over disk	Transdermal drug products.
Apparatus VI	Cylinder	Transdermal drug products.
Apparatus VII	Reciprocating disk	Extended-release drug products

5. Fit factor

5.1 Similarity factor f2 :

The US FDA emphasises similarity and difference variables when comparing *in-vitro* dissolution patterns. Similarity factor (f2) emphasises the comparison of the similarity of two comparable formulations, as the name implies. To determine whether two dissolution profiles are similar, the f2 parameter is frequently utilised.The FDA defines 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". The similarity factor calculation formula is as follows :

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

5.2 Dissimilarity factor (f1):

The dissimilarity factor is concerned with the variation in % dissolved between the reference and test at different times. As a result, the parameters directly contrast the variance between a test product's and a reference product's percent medication dissolved per unit time. To estimate the percentage of inaccuracy in the medication release profile, utilise the f1 factor. Ideally, f1 will range from 0 to 15[12]. The difference factor f1 is specified as

$$f1= \{[\Sigma\ t=1\ n\ |Rt-Tt|] / [\Sigma\ t=1\ nRt]\} \times 100.$$

The criteria stated by US FDA for dissolution profile are as follows:

1. Only when a total of 12 or more dissolution units are used can the dissolution profiles be compared. The average mean dissolution data of 12 units should be used to calculate f2.
2. A statistical method of establishing confidence intervals to assess whether the reference and test are statistically significant or not may be used to calculate the similarity factor accurately.
3. The dissolution circumstances, such as dosage form strength, test time intervals, temperature, rpm, and total test time, should be the same for the reference and test items.
4. Since f2 values are sensitive to the amount of dissolving time points, the literature likewise advises just taking into account one time after 85% product dissolution.
5. Comparison of dissolution patterns is not required for compounds with quick dissolution, which may dissolve 85% in 15 minutes.
6. A similarity factor of 50 to 100 guarantees that two goods are identical.
7. A difference factor of 0 to 15 guarantees slight variations between two items.

6. *In vitro- in vivo* correlation (IVIVC)

In vitro in vivo correlation, or IVIVC, is the establishing of a relationship between a drug's or dosage forms in vitro properties (such as release profile or dissolution profile) and in vivo properties (such as absorption profile). IVIVC is a biopharmaceutical tool that can speed up drug development, decrease the need for in vivo testing, cut drug development expenses, and raise product quality[14]. Dissolution parameters can be established using IVIVC, and the clinical applicability of in vitro dissolution can be evaluated. In vitro in vivo relationships, or IVIVRs, are frequently used to describe nonlinear methods. It includes any kind of connection between in vitro characteristics and in vivo performance that is not covered by the traditional IVIVC notion previously stated. A dissolution safe space, for instance, results when changes in in vitro dissolution qualities do not affect in vivo performance. The FDA defines three basic tiers based on the type of evidence required to establish the relationship[15]:

Level A - The point-to-point link between in vitro dissolution and the in vivo input rate (for example, the in vivo dissolution of the drug from the dosage form) is represented by this type of correlation, which is often linear. The in vitro dissolution and in vivo input curves in a linear correlation may be directly superimposed or may be made to be superimposed by the use of a scaling factor. Although uncommon, nonlinear correlations may also be useful.

Level B -The statistical moment analysis principles are used by A Level B IVIVC. Either the mean residence time or the mean in vivo dissolution time are used to compare the mean in vitro dissolution time. Because several different in vivo curves will result in values for the mean residence time that are similar, a Level B correlation does not accurately reflect the actual in vivo plasma level curve.

Level C - A Level C IVIVC provides a single point relationship between a pharmacokinetic parameter (e.g., AUC, Cmax, or Tmax) and a dissolution parameter (e.g., t50%, percent dissolved in 4 hours). The shape of the plasma concentration-time curve, which is the key element defining the performance of ER products, is not fully reflected by a Level C correlation.

7. Example of *in vitro* Bioequivalence Studies

7.1 *In vitro* Bioequivalence Study of Paracetamol Tablets

Preparations:

Three different 500 mg paracetamol tab products made in Mexico were investigated: Tempra lot FDE16 from Mead Johnson, Mexico; Tylenol 22273 from Cilag Mexico; Febrim lot 2138 from Rimsa Mexico; and Tylenol lot JBA145 from McNeil, Fort Washington, PA, which served as the reference product (innovator). C, D, E, and I (innovator) were the letters assigned at random to identify each product.

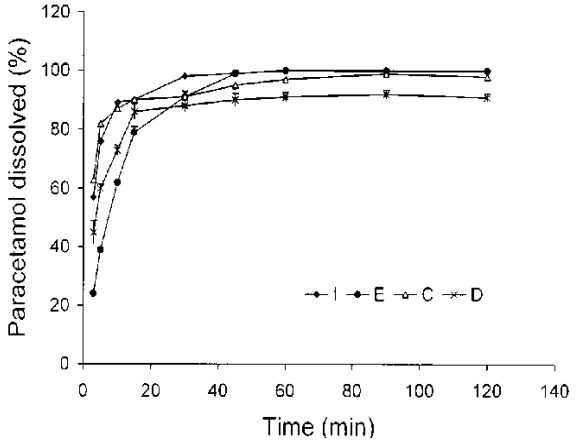
***In-vitro* Research:**

Twenty tablets of each brand were subjected to weight variation, content, and content uniformity assays in accordance with the USP 23 protocol for paracetamol tablets (U.S. Pharmacopeial Convention, The United States Pharmacopoeia 23, Author, Rockville, MD).

The USP 23 technique was used on 12 subjects to investigate the release characteristics of the paracetamol Mexican products and the innovator product (500 mg tablets). 5 ml were taken out of the filtered samples at intervals of 1, 3, 5, 10, 15, 30, 45, 60, 90, and 120 minutes. Dissolution profiles from commercial paracetamol products were evaluated spectrophotometrically at 242 nm using a previously validated method (A.M. Dominguez and M. Hurtado, Dissolution profiles from commercial paracetamol products, Rev. Mex. C Farm., 27(1-2):13-19). Samples were diluted with the dissolution medium (phosphate buffer at pH 5.8). A calibration curve that was created on the same day as the trial was used to compare the amount of paracetamol dissolved at each time[16].

Protocol for *in-vivo* research

Twelve healthy volunteers—six men and six women, ages 21 to 26; weights of 48 to 72 kg; and heights of 160 to 172 cm—were used for the in vivo tests. According to results from physical exams, haematological testing, and urine tests, all volunteers were in good physical health. Each individual provided written consent to participate after being told of the study's goal, protocol, and risks. The subjects abstained from alcohol and other drugs for at least two weeks prior to the study's start date. Each of the four items was administered to each subject in accordance with a complete crossover Latin square design (4 4) with balance for the first residual effect. Subjects were assigned at random. Prior to the experiment, each subject fasted for at least one night. Following the dose, food was restricted for four hours. 150 cc of water were consumed along with the pills. To ensure appropriate hydration, each participant also drank 300 ml of water two hours before the medication was administered, as well as 150 ml of water at 1, 2, 3, and four hours after treatment. All subjects received a regular breakfast 4 hours after dose, followed by a standard lunch 4 hours later. This process was repeated until all of the products were administered every week. There was a week-long washout time between the administration of each product[18].



Following USP 23 dissolution test specifications for paracetamol tablets, dissolution profiles were created for generic (C, D, E) and innovator (I) paracetamol tablet products. The data are the mean across a sample of 12 tablets plus or minus the standard error. Urinary Excretion: Before administering the drug, volunteers were asked to provide blank urine samples. Each of the following time periods saw the collection of quantitative urine samples: 0 0.5 0.5 1.0 1.5 2.0 3.0 4.0 6.0 8.0 10.0 12.0 12.0 24.0 hours following dosage. Amounts of each sample were divided into equal parts and frozen in labelled containers until the day of

analysis. The total volume of urine passed during each time period was calculated. Following USP 23 dissolution test specifications for paracetamol tablets, dissolution profiles were created for generic (C, D, E) and innovator (I) paracetamol tablet products. The data are the mean across a sample of 12 tablets plus or minus the standard error. Urinary Excretion: Before administering the drug, volunteers were asked to provide blank urine samples. Each of the following time periods saw the collection of quantitative urine samples: 0 0.5 0.5 1.0 1.5 2.0 3.0 4.0 6.0 8.0 10.0 12.0 12.0 24.0 hours following dosage. Amounts of each sample were divided into equal parts and frozen in labelled containers until the day of analysis. The total volume of urine passed during each time period was calculated[19].

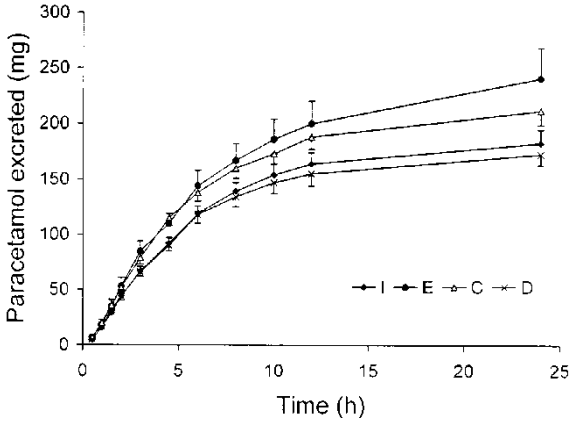
***In-vitro* Research:**

When it came to weight variation, content test, and content uniformity assay, every product complied with pharmacopoeia requirements. Fig. 1 displays the dissolution patterns of the four brands that were investigated. The values are the mean (plus standard error) of 12 units. Although there were significant differences in the rates of dissolution across the entire profile, all tablets dissolved within the USP 23 dissolution requirements (not less than 85% of the labelled amount of paracetamol dissolved in 30 minutes). In comparison to the innovator product, products C and E demonstrated faster dissolution rates, particularly in the first 30 minutes. Compared to the other products, product D's total amount of paracetamol dissolved at 120 minutes showed significant differences. The logarithm of the plot of the remaining percentage to be dissolved versus time was used to calculate the dissolution rate constants, assuming first-order kinetics for fast dissolving products (J. G. Wagner, Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm. Sci., 58:1253). The profiles found show that for fast dissolving compounds, dissolution is related to apparent first-order kinetics. These findings support those made by Najib and Jalal, who noted first-order dissolution kinetics in the case of fast-release paracetamol tablets (J. G. Wagner, Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm. Sci., 58:1253). The logarithm of the plot of the remaining percentage to be dissolved versus time was used to calculate the dissolution rate constants, assuming first-order kinetics for fast dissolving products (J. G. Wagner, Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm. Sci., 58:1253). The profiles found show that for fast dissolving compounds, dissolution is related to apparent first-order kinetics. These findings support those made by Najib and Jalal, who noted first-order dissolution kinetics in the case of fast-release paracetamol tablets (J. G. Wagner, Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules, J. Pharm. Sci., 58:1253)[20,21].

Both the dissolution t50 and t85 values were computed. The t50 in this study is consistent with all experimentally acquired data for all items. The items examined in the current investigation had statistically different t50 and t85 values (p .05), therefore they could not be compared with respect to their in vitro release properties.

***In-vivo* Research:**

As a gauge of bioavailability, the total amount of paracetamol excreted (free plus conjugated) in urine was used. Although a plasma method for paracetamol and its metabolites exists, using a noninvasive method (urine method) might allow us to quickly and easily distinguish between the bioavailability characteristics of different paracetamol generic products. In many cases, the best way to estimate bioavailability is by analysing drug blood levels. so this research might be considered as an alternative for ‘‘screening’’ bioequivalence properties of paracetamol commercial products existing in the Mexican market. Since urinary data were deemed sufficient to evaluate variations in the absorption of paracetamol from generic drug products, it has been well established that urinary excretion rates of paracetamol are directly proportional to serum concentrations (G. L. Mattok, I. J. Mc Gilveray, and C. A. Mainville, Acetaminophen III: dissolution studies of commercial tablets of acetaminophen and comparison with in vivo absorption parameters, J. Pharm[22].



Mean cumulative amount of paracetamol excreted in urine after oral administration of paracetamol

tablet products. Data represent the mean of 12 subjects plus or minus standard error. A single in vitro-in vivo correlation for immediate-release medications may be able to predict the efficacy of numerous formulations when tested in vivo. For instance, a single in vivo pharmacokinetic measure like AUC, Cmax, Xut, or anything similar may be associated with in vitro MRT. There are no research for paracetamol tablets on the association of the in vivo bioavailability parameters and the official dissolving test, despite the fact that the dissolution test was developed in 1971 in USP 21. By using three straightforward dissolving techniques, Mattok et al. evaluated the breakdown of several batches of paracetamol tablets. When assessed from blood or urine profiles, none of the approaches offered a perfect connection with the physiological availability of paracetamol.

However, Evora et al. found discrepancies between the in vitro results obtained after the pharmacopoeia dissolution test and the bioavailability shown with a tablet formulation (C. M. Evora, E. Sanchez, and M. Llabre's, Discrepancy between pharmacopoeia dissolution test and bioavailability of paracetamol tablets, Il Farmaco, 45:569)[23]. When using dissolution conditions other than those required by the USP method, Sotiropoulus et al. suggested[24] comparing the amount of paracetamol recovered in urine after drug administration and the dissolution parameters.

8. Applications of Bioequivalence studies with *in vitro-in vivo* correlation

Bioequivalence studies with in vitro-in vivo correlation can be a helpful tool in the development and regulation of drugs in a number of ways[25], including:

(i) Early identification of possible problems: *In vitro* investigations can aid in the early detection of potential problems with drug formulations, which can assist save time and resources in later-stage clinical trials.

(ii) Better drug design: By highlighting the key pharmacological characteristics that influence *in vivo* performance, *in vitro-in vivo* correlation studies can aid enhance drug design. Drug development that is economical: In vitro testing is often less expensive than in vivo testing and can eliminate the necessity for pricey animal experiments.

(iii) Regulatory compliance: *In vitro-in vivo* correlation studies can assist assure compliance with these requirements since regulatory bodies need bioequivalence studies to authorise generic medications.

(iv)Quicker drug approval: By supplying information that may be used to inform regulatory decisions, in vitro-in vivo correlation studies can hasten the licencing of new medications. In general, including *in vitro-in vivo* correlation into bioequivalence studies can aid in enhancing drug development effectiveness, cutting costs, and ensuring regulatory compliance.

9. Advances in Bio Equivalence Studies for ophthalmic medications

BE research is costly, time-consuming, and difficult to conduct since it calls for taking at least 10–20 blood samples over an extended period of time from a number of healthy volunteers using an indwelling catheter. After the washout period, a similar procedure is repeated in the volunteers using the test/reference products. Additionally, all blood samples taken should have their drug concentrations measured, and the results should then be statistically analysed using pharmacokinetic software. Depending on the dose type, a drug's release in the body varies. medications delivered intravenously are regarded as being entirely bioavailable, but medications taken orally may only be partly absorbed and metabolised. The majority of eye drugs come in conventional dose forms such solutions, ointments[26, 27].

10. Problems associated with ophthalmic medications bioequivalence testing[28] -

1. Despite limitations such quick removal from the precorneal region and inadequate ocular absorption, ophthalmic solutions nevertheless exceed suspensions.
2. Suspension dosage forms are taken into account when the medications have a low water solubility and are hydrophobic.
3. Despite using a large number of enrolled participants, formulation changes cannot be demonstrated to be efficacious due to the modest dose of the formulation (one to two drops) and subjective variability in pathophysiological factors that greatly contribute to the data variability.
4. The physical stability and ocular bioavailability of the ophthalmic product are greatly influenced by the particle size of the product.
5. The inflow and outflow of lacrimal fluids cause variations in the drug's intrinsic dissolving rate.
6. Due to physicochemical differences, even equivalent solutions or suspensions with equal active and inert components

Advances done to overcome Problems associated with ophthalmic medications bioequivalence testing[29] –

1. To increase patient access to generic drugs, academia, business, and regulatory organisations have been attempting to create new methods for proving the BE of topical products that are quicker, more accurate, and more repeatable.
2. The U.S. FDA has pushed the identification of critical quality characteristics (CQA) and the development of pertinent IVRT/*in-vivo* product performance tests for the assessment of bioequivalence through the Generic Drug User Fee Amendments (GDUFA) agreement with the generic industry.
3. To prove the 'sameness' between RLD and test product, the FDA has released guidance guidelines for certain goods offering *in-vitro* (physicochemical characteristics and IVRT) and *in-vivo* (clinical endpoint research) choices.

Product Type	**Criteria for *in-vitro* option**	**Criteria for *in-vivo* option**
Ophthalmic Solution	• Viscosity • Specific gravity • Buffer capacity • pH • Osmolality	• Bioequivalence study with pharmacokinetic endpoints
Ophthalmic Suspensions/ Emulsions	• Viscosity • Surface tension • Osmolality • Globular size distribution • pH • The soluble fraction of the drug • Drug substance properties • Zeta potential • IVRT a. USP IV b. USP II c. Franz diffusion cells	• Bioequivalence study with a pharmacokinetic endpoint • Aqueous humor pharmacokinetic studies
Ophthalmic Ointments	• Appearance • Polymorphic form • Acidity & alkalinity of the extracted ointment base • Yield stress • Viscosity • Drug particle size and size distribution • *In-vitro* microbial kill rate study against 18 organisms (if the product contains antibiotic) • IVRT studies a. USP IV b. USP II c. Franz diffusion cells d. Float-a-lyzer dialysis	• Bioequivalence study with a pharmacokinetic endpoint • Aqueous humour pharmacokinetic studies
Ophthalmic Gel	• pH • Specific gravity • Osmolality • Soluble fraction • Yield stress • Viscosity • Drug particle size distribution • IVRT a. USP IV b. USP II c. Franz diffusion cells d. Float-a-lyzer dialysis	• Bioequivalence study with pharmacokinetic endpoints

Therefore, for each tested lot, these measurements must be made in triplicate. A minimum of three display batch lots of the test product and undistributed commercial lots of the reference product should be used for testing. It is clear from the PSGs available for ophthalmic solutions, suspensions, emulsions, ointments, and gels to obtain a biowaiver that the test product's physicochemical properties (pH, osmolality, specific gravity, buffer capacity, tonicity, and viscosity) should be comparable to the RLD in addition to its qualitative and quantitative sameness.

When requesting a waiver for any generic drug products or for BE recommendations, agencies can be consulted because their PSG databases are promptly updated.

11. Conclusion:

IVIVC is a crucial instrument in the research and regulation of pharmaceuticals. IVIVC can be used to anticipate a medication's in vivo behaviour from in vitro data, which might result in more effective drug development, lower costs, and quicker regulatory clearance. Early in the development phase, IVIVC may be used to spot possible problems with medication formulations, enabling prompt interventions to improve drug design. Due to the fact that in vitro testing is typically less costly and more scalable, it may also be utilised to decrease the need for animal testing.

IVIVC is also a significant regulatory criteria for the registration of generic medications. IVIVC can show that generic medications are equal to their branded equivalents in terms of safety and efficacy by establishing a link between in vitro and in vivo performance. IVIVC is a crucial instrument for drug development and regulation overall, offering insightful information on drug behaviour and streamlining the drug development procedure.

Acknowledgments

The authors thank SGT College of Pharmacy, Shree Guru Gobind Singh Tricentenary (SGT) University, Gurugram, Haryana India for technical support.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Midha KK, McKay G. Editorial: Bioequivalence; its history, practice, and future. Vol. 11, AAPS Journal. 2009. p. 664–70.
2. Setiawati A. The importance of bioequivalence study: focus on clopidogrel. Vol. 20. 2011.
3. Abbirami V, Sainithya P, Shobana A, Ramya Devi D, Vedha Hari B. A Review on In-vitro Bioequivalence Studies and its Methodologies. Vol. 5, International Journal of ChemTech Research CODEN(USA.
4. Chen ML, Shah V, Patnaik R, Adams W, Hussain A, Conner D, Mehta M, Malinowski H, Lazor J, Huang SM, Hare D. Bioavailability and bioequivalence: an FDA regulatory overview. Pharmaceutical research. 2001 Dec;18:1645-50.
5. Sarkar A. Types of Biowaivers: A Discussion. International Journal of Drug Regulatory Affairs. 2019 Sep 15;7(3):14–20.
6. Williams RL, Adams WP, Poochikian G, Hauck WW. Content uniformity and dose uniformity: current approaches, statistical analyses, and presentation of an alternative approach, with special reference to oral inhalation and nasal drug products. Pharmaceutical research. 2002 Apr;19:359-67. 7. Zakeri-Milani P, Nayyeri-Maleki P, Ghanbarzadeh S, Nemati M, Valizadeh H. In-vitro bioequivalence study of 8 brands of metformin tablets in Iran market. Journal of Applied Pharmaceutical Science. 2012 Aug 30;2(8):194-7.
7. Blanco M, Alcalá M. Content uniformity and tablet hardness testing of intact pharmaceutical tablets by near infrared spectroscopy: a contribution to process analytical technologies. Analytica chimica acta. 2006 Jan 31;557(1-2):353-9.
8. Seitz JA, Flessland GM. Evaluation of the physical properties of compressed tablets I: Tablet hardness and friability. Journal of Pharmaceutical Sciences. 1965 Sep 1;54(9): 1353-7.
9. Abdelbary G, Eouani C, Prinderre P, Joachim J, Reynier JP, Piccerelle PH. Determination of the in vitro disintegration profile of rapidly disintegrating tablets and correlation with oral disintegration. International journal of pharmaceutics. 2005 Mar 23;292(1-2):29-41.
10. Uddin R, Saffoon N, Sutradhar KB. Dissolution and dissolution apparatus: a review. Int J Cur Biomed Phar Res. 2011;1(4):201-7.
11. Suarez-Sharp S, Abend A, Hoffelder T, Leblond D, Delvadia P, Kovacs E, Diaz DA. In vitro dissolution profiles similarity assessment in support of drug product quality: what, how, when—workshop summary report. The AAPS Journal. 2020 Jul;22:1-2.
12. Muselík J, Komersová A, Kubová K, Matzick K, Skalická B. A critical overview of FDA and EMA statistical methods to compare in vitro drug dissolution profiles of pharmaceutical products. Pharmaceutics. 2021 Oct 15;13 (10): 1703.
13. Emami J. In vitro-in vivo correlation: from theory to applications. J Pharm Pharm Sci. 2006 Apr;9(2):169-89.
14. Lu Y, Kim S, Park K. In vitro–in vivo correlation: Perspectives on model development . International journal of pharmaceutics. 2011 Oct 10;418(1):142-8.
15. Domínguez AR, Medina RL, Hurtado MP. Bioequivalence study of paracetamol tablets: In vitro-in vivo correlation. Drug Dev Ind Pharm. 2000;26(8):821–8.
16. Domínguez R A, Medina RL. Bioequivalence study of paracetamol tablets: in vitro-in vivo correlation. Drug development and industrial pharmacy. 2000 Jan 1;26(8):821-8.
17. Li D, Xu S, Wang Y, Li D, Li X, Pan J, Xu P. Pharmacokinetics and drug-drug interaction between enalapril, enalaprilat and felodipine

extended release (ER) in healthy subjects. Oncotarget. 2017 Sep 9;8(41):70752.
18. Emara LH, Emam MF, Taha NF, El-ashmawy AA, Mursi NM. In-vitro dissolution study of meloxicam immediate release products using flow through cell (USP apparatus 4) under different operational conditions. Int J Pharm Pharm Sci. 2014 Oct;6(11):254-60.
19. Wagner JG. Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. Journal of pharmaceutical sciences. 1969 Oct 1;58 (10): 1253-7.
20. Khan KA. The concept of dissolution efficiency. Journal of pharmacy and pharmacology. 1975 Jan;27(1):48-9.
21. Mattok GL, McGilveray IJ, Mainville CA. Acetaminophen III: Dissolution studies of commercial tablets of acetaminophen and comparison with in vivo absorption parameters. Journal of Pharmaceutical Sciences. 1971 Apr 1;60(4):561-4.
22. Domínguez R A, Medina RL. Bioequivalence study of paracetamol tablets: in vitro-in vivo correlation. Drug development and industrial pharmacy. 2000 Jan 1;26(8):821-8.
23. Sotiropoulus JB, Deutsch T, Plakogiannisx FM. Comparative bioavailability of three commercial acetaminophen tablets. Journal of Pharmaceutical Sciences. 1981 Apr 1;70(4): 422-5.
24. Kaur P, Jiang X, Duan J, Stier E. Applications of in vitro–in vivo correlations in generic drug development: case studies. The AAPS journal. 2015 Jul;17:1035-9.
25. Renukuntla J, Palakurthi SS, Bolla PK, Clark BA, Boddu SH, Manda P, Sockwell S, Charbe NB, Palakurthi S. Advances in In-vitro bioequivalence testing methods for complex ophthalmic generic products. International Journal of Pharmaceutics. 2022 Sep 24:122209.
26. Chockalingam A, Xu L, Stewart S, LeMerdy M, Tsakalozou E, Fan J, Patel V, Rouse R. Protocol for evaluation of topical ophthalmic drug products in different compartments of fresh eye tissues in a rabbit model. Journal of Pharmacological and Toxicological Methods. 2019 Mar 1;96:9-14.
27. Myoenzono A, Kuribayashi R, Yamaguchi T, Ogawa T, Takagi K. Current regulation for bioequivalence evaluations of generic ophthalmic dosage forms in Japan. European Journal of Drug Metabolism and Pharmacokinetics. 2020 Dec;45:697-702.
28. Choi SH, Lionberger RA. Clinical, pharmacokinetic, and in vitro studies to support bioequivalence of ophthalmic drug products. The AAPS journal. 2016 Jul;18:1032-8.
29. Shah VP, Yacobi A, Rădulescu FŞ, Miron DS, Lane ME. A science based approach to topical drug classification system (TCS). International journal of pharmaceutics. 2015 Aug 1;491(1-2):21-5.