Comparative Bioavailability and *in vitro* Characterization of two Brands of Diclofenac sodium

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**DECLARATION**

I hereby declare that the project work entitled as **“Comparative bioavailability and in vitro characterization of two brands of Diclofenac sodium”** submitted to Department of Pharmaceutical Sciences, Hemwati Nandan Bahuguna Garhwal University (A Central University) Srinagar, Garhwal is a project work done.

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**CERTIFICATE**

This is to certify that the project report entitled Comparative Bioavailability and in vitro Characterization of two Brands of Diclofenac Sodium has been prepared by me for BP813PW Course in VIII Semester. Such materials as obtained from other sources have been duly acknowledged in the project report

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Comparative Bioavailability and *in vitro* Characterization of two Brands of Diclofenac Sodium

# 1. Introduction

The term "*in vitro* bioavailability assessment" describes the study of a drug's availability and release rate from a pharmaceutical dosage form (such as tablets, capsules, or patches) utilising laboratory tests and simulated settings. Its goal is to reveal how much of the medication is liberated from the dose form and how quickly it becomes accessible for absorption into the systemic circulation.

Different dissolution and release testing techniques are used during *in vitro* bioavailability assessment to mimic the conditions the drug would experience in the human body. These techniques usually replicate the pH and make-up of gastrointestinal fluids. Since dissolution is a crucial step in the process of a medicine being absorbed into the bloodstream, the dissolution testing technique evaluates the rate at which the drug is dissolved from the dosage form.

## **1.1 Significance of study:**

Studies on *in vitro* bioavailability are essential for developing new pharmaceuticals. The rate and extent to which an active ingredient or medication is absorbed into the systemic circulation and made available at the site of action are referred to as bioavailability. It is a crucial factor in figuring out a drug's efficacy and therapeutic potential.

The following are a few major implications of *in vitro* bioavailability studies:

* Early drugs Screening: Early in the process of discovering and developing a medicine, *in vitro* bioavailability studies are frequently carried out. They aid in the evaluation of the drug's permeability across biological barriers like the skin and gastrointestinal system as well as its solubility y and rate of dissolution. By being aware of these variables, scientists can see any problems that can impair the bioavailability of the medication, make the required adjustments, or quickly rule out candidates who aren't a good fit.
* *In vitro* bioavailability studies offer important information for designing pharmaceutical drugs. Researchers can improve the formulation to increase bioavailability by examining elements like the drug's solubility and dissolving behaviour. The drug's solubility and subsequently its absorption can be improved, for example, by altering the drug's particle size, utilising various excipients, or using

unique delivery systems.

* Cost- and time-efficient alternatives to in vivo research include *in vitro* bioavailability studies, which can be carried out in a controlled laboratory setting. These investigations aid in the prioritisation of attractive candidates, the reduction of animal or human trials, and the cost-effective use of resources by giving an early indication of a drug's potential for absorption.
* Drugs are categorised using the biopharmaceutical classification system (BCS) according to their solubility and permeability properties. Data from *in vitro* bioavailability tests is used to assign a drug's BCS classification. In order for regulatory authorities and pharmaceutical scientists to make educated decisions on the development and regulatory requirements of a drug product, this classification is helpful in indicating the drug's behaviour in vivo.

*In vitro* bioavailability studies are essential for quality control during the production of pharmaceutical products. Pharmaceutical businesses can guarantee batch-to-batch uniformity and product quality by developing repeatable and consistent procedures to evaluate bioavailability. Through the manufacturing process, these studies aid in maintaining the optimal bioavailability of medication formulations.

## **1.2 Diclofenac sodium:**

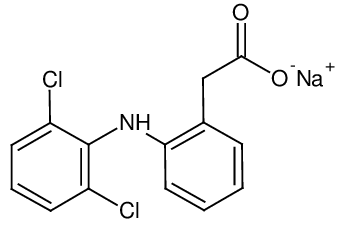
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Figure 1 Chemical structure of Diclofenac sodium

**Chemical formula:**C14H10Cl2NNaO2

**Chemical name:** 2-[(2,6-dichlorophenyl)-amino] phenyl acetate.

**Molecular weight:** 318.1

**Dose:** Orally or by intramuscular injection 25 to 75 mg.

**Description:** A white to slightly yellowish crystalline powder slightly hygroscopic.

Diclofenac sodium is a nonsteroidal anti-inflammatory drug (NSAID) that is frequently used to treat different medical diseases (rheumatoid arthritis and osteoarthritis) and relieve pain and inflammation. It is a member of the group of drugs called phenyl acetic acid derivatives. Diclofenac sodium functions by preventing the body from producing particular molecules known as prostaglandins. The substances called prostaglandins are the source of fever, inflammation, and discomfort. Diclofenac sodium helps relieve pain and inflammation brought on by disorders including arthritis, menstrual cramps, migraines, and other inflammatory conditions by lowering the production of these chemicals.

# 2. Objective:

Objective is to learn and compare the dissolution characteristics, release profile of various brands of diclofenac sodium. To measure the rate and volume of medication release from each brand and examine any potential variations in bioavailability that might affect the therapeutic efficacy and clinical outcomes related to their use

# 3. Regulatory requirement:

Following the rules and techniques described in the Pharmacopoeia is necessary to conduct an *in vitro* bioavailability test of a medicine in accordance with the pharmacopeia. The provides the following general instructions for conducting an *in vitro* bioavailability test:

* Get instruct with the monograph: Every medication listed in the has a monograph that offers detailed guidelines for carrying out experiments, such as *in vitro* bioavailability studies. Find the diclofenac sodium’s monograph.
* Choose the best *in vitro* techniques: The may list the suggested *in vitro* techniques for determining medication bioavailability. These techniques may consist of permeation investigations, dissolution tests, or other pertinent procedures. The precise approach or methods to be used will be laid out in the monograph.
* Create the test systems or solutions: Follow the guidelines in the monograph while preparing the test solutions or systems.
* Execute the *in vitro* testing: Execute the suggested *in vitro* tests in accordance with the instructions provided in the monograph.
* Analyse measurements or sample data: Take samples or measurements as the monograph instructs or at predetermined intervals of time. Use proven analytical techniques to analyse the samples or data in order to find out the medication concentrations, dissolution profiles.
* Assess the *in vitro* bioavailability of the medicine by comparing the acquired results to the reference standards or criteria provided in the monograph. Verify that the acceptance standards described in the monograph are being followed.
* Record the findings and present them: Keep track of all pertinent experimental information, observations, and findings from the *in vitro* bioavailability testing. Create a thorough report that includes a summary of the procedures, findings, and recommendations.

It is crucial to carefully adhere to the recommendations and instructions given in the drug's monograph. To promote consistency and standardisation in testing, the includes detailed instructions.

# 4. Experimental requirement:

**Chemicals:**

* Base drug
* Cipzen-D®(Branded) and Voltaren®(Generic)
* Potassium dihydrogen phosphate (KH2PO4)
* Distilled water
* Sodium hydroxide (NaOH)

**Apparatus/ Glassware:**

* Dissolution test apparatus
* UV spectrometer
* Volumetric flask
* Test tubes
* Test tube holder
* Conical Flask
* Filter funnel
* Filter paper
* Sterile Syringe (5ml)

# 5. Experimental Methodology:

## **5.1 Preparation of buffer:**

* Potassium dihydrogen phosphate (KH2PO4) should be carefully weighed at 27.218 grams and combined with 1000 ml of distilled water.
* In an another flask, weigh precisely 8 grams of sodium hydroxide and combine it with 1000 ml of distilled water.
* Mark both the flasks with their chemical name.
* Now combine 250 ml of potassium dihydrogen phosphate solution, 195.5 ml of sodium hydroxide solution, and 550 ml of distilled water in a one-litre volumetric flask. Stir thoroughly.
* This will prepare the 1 litre of phosphate buffer saline 7.4.
* Repeat the procedure to make another litre of phosphate buffer saline.

## **5.2 Preparation of Stock sample for calibration curve:**

* Combine 10 grams of the free drug with 100 ml of distilled water, this will be **stock** **sample 1** of 1000µg
* Now take 10 ml from stock sample 1 and add 90 ml of distilled water this will be **stock** **sample 2** of 100µg.
* Further take 10ml from stock sample 2 and 90 ml of distilled water this will be **stock** **sample 3** of 10µg.
* Next, add 8 mL of distilled water to 2 mL of stock sample 3; repeat with 4 mL, 6 mL, and 8 mL of sample; and 6 mL, 4 mL, and 2 mL of distilled water; then mark the test tubes.

# 6. Dissolution Test:

This test is intended to ascertain whether the requirements for solid dose delivered orally are being complied with. The test is designed for a pill or capsule. This test is offered to ascertain whether solid dose forms taken orally are in conformity with the dissolution requirements.

All metallic components of the apparatus that might come into contact with the preparation or the dissolution medium must be made of stainless steel or an equivalent material, or they must be coated with a suitable substance to prevent reaction or interference with the preparation being tested or the dissolution medium.

**Dissolution Apparatus:**

Figure 2 Dissolution Apparatus

An Assembly consist of the following:

1. A cylindrical vessel with an internal diameter of 98–106 mm made of borosilicate glass or another suitable transparent material, with a hemispherical bottom and a notional capacity of 1000 ml. The jar features a flanged upper rim and a lid with several apertures, one of which is in the centre, to prevent evaporation. Vessels with a nominal capacity of 150 ml, 2000 ml, and 4000 ml are required for some applications. Coning can be eliminated using modified peak vessels. Examine criteria such as hemisphere radius (49 mm to 53 mm), cylinder roundness, cylinder perpendicularity, hemisphere roundness, and cylinder/hemisphere concentricity in addition to vessel parameters such inner diameter and height.
2. A motor with a speed regulator that can keep the serialised basket’s rotational speed within 4% of the individual monograph's set value. The motor has a stirring element installed that is made up of a drive shaft and basket.
3. The metal shaft turns without noticeable wobble and with ease. There are two parts to the basket. The top part, which has a vent, is attached to the shaft C and has three spring cls—or other suitable fasteners, such as O-rings—that enable the lower part of the basket to be removed for the introduction of the preparation being examined and that firmly hold the lower part of the basket concentric with the axis of the vessel during rotation. The basket's lower, detachable portion is composed of welded-steam fabric with square perforations measuring 0.381 mm in size and wire thickness of 0.254 mm. It is shaped into a cylinder with a narrow sheet metal rim around the top and bottom. a. The stirring element's shaft and basket are made of stainless steel or another inert material. Throughout the test, the distance between the basket and the interior of the vessel is kept constant at 23 to 27 mm. Frequently check the integrity of the basket mesh with a microscope or magnifying lens.
4. A heating jacket or any other appropriate heating equipment that keeps the dissolving media at 36.5° to 37.5°. Throughout the test, the bath liquid is kept moving smoothly and continuously. The vessel is tightly clamped in the water-bath to reduce displacement vibration caused by other equipment, such as the water circulation system.

## **6.1 Types of dissolution apparatus:**

1. **Basket type:**

It is made of borosilicate glass and has a 1000 ml maximum capacity. The shaft is composed of stainless steel, and the shape is semi-hemispherical at the bottom. The cylinder basket is secured by the shaft. Because it rotates smoothly and must match the suggested USP, it is frequently referred to as a rotating basket. 100 rpm is the typical speed limit. It is utilized for delayed release, floating dose forms, suppositories, and capsules or tablets.

1. **Paddle type**

This equipment is specifically designed, and it has a coated paddle that lessens the disturbance caused by the churning. It appears to have a that makes touch with the shaft's bottom. The Paddle device is made of stainless steel. The platinum wire on it also prevents the capsules from floating. The paddle is kept at 37 C and the motor speed is typically set to 40. The paddle is retained in the position that is required by the current USP; for capsules, the motor speed is 50 rpm, while for suspensions, it is 25 rpm.

1. **Reciprocating Cylinder:**

Typically, this dissolution apparatus is taken into account while developing controlled release preparations. This is due to the GI tracts' ability to release products after being subjected to varied mechanical and physicochemical conditions. It is a simple method for testing for drugs, and there are no issues with the PH values of its solutions. Chewable pills with prolonged release are made with it.

1. **Flow through the cell:**

The open system and the closed system are the two types that make it up. Fresh dissolving medium is pumped into the cells of the open system before the fractions are added. Every 30 minutes, the fractions are usually drawn. The most ideal sink conditions should be used for the dissolving test using equipment. The dissolving gas is injected into the circle in a closed system, on the other hand, and no new medium is added. The test is carried out in small amounts and is often utilized for medications with modest dosages. Implants frequently utilise the flow via the cell machinery, which is fashioned like a reservoir.

1. **Paddle over the disk:**

In order to hold the product so that the surface can be levelled with the paddle, it has a shaft and a disk assembly. The disk assembly and the pad were attached. It has a 900 ml volume capacity.

1. **Rotating cylinder:**

It also has a stainless steel cylinder and uses a vessel rather than a basket cylinder. On cuprophan, the device is positioned to follow the cylinder. The dosage amount is put within the cylinder, where it will be removed into the water bath from the outside. The issue with this pharmaceutical drug testing dissolution is that the transdermal patches cannot be reduced in size.

1. **Reciprocating disk:**

This device has a 50–200 ml volume capacity flat-bottomed cylindrical vessel. The dose quantity is extracted in a water bath, and it is often mounted on a disk-shaped holder that also manufactures transdermal patches. Only modest dosages are used for controlled release creation.

## **6.2 Dissolution medium:**

Use the dissolve agent that is listed in the specific monograph. If the medium is a buffered solution, the solution should be adjusted to have a pH that is within 0.05 units of the monograph's recommended pH. Prior to testing, the dissolving medium needs to be deaerated. By comparing the concentration of dissolved oxygen before and after deaeration, you may gauge the degree of deaeration.

Steps to perform Dissolution Test:

* Mark up to 900ml of phosphate buffer saline in each of the two cylindrical vessels.
* Place both (generic and branded) tablets in separate baskets, then close them.
* Attach the basket to the rotating metal shaft.
* Once you push the machine's "start and down" button, it will begin to rotate at 100 RPM and descend in a buffer. Now the dissolution starts…
* After the five minutes are up, use a syringe to take the five millilitres of sample from each cylindrical vessel, place them in a different test tube, and then add the five millilitres of buffer right away. Repeat the same steps every 10,20,30,40,50,60,70,80 and 90 minutes.

# 7. Preparation of calibration curve using UV spectrometer

A number of procedures must be followed in order to establish the correlation between drug concentration and UV absorbance measured using a UV spectrometer to create a calibration curve for medications. This curve is crucial for calculating the drug concentration in samples whose absorbance values are unknown. The procedures for producing a calibration curve with a UV spectrometer are listed below:

**Step 1: Preparation of standard solution:**

A pure sample of drug diclofenac sodium obtained from department of pharmaceutical sciences. It was highly pure and precisely weighed. Prepared standard solutions in a range of concentrations 2µg to 100 µg. Solutions with various concentrations 2 µg, 4 µg, 6 µg, 8 µg, 10 µg, 12 µg, 14 µg, 6 µg, 18 µg, 20 µg, 100 µg for calibration curve. The appropriate range of drug concentrations samples covered by these concentrations.

To get these concentrations, dilute the pure drug material in an organic solvent that is methanol with the help of volumetric flask.

**Step 2: UV spectrometer setup:**

Turned on the UV spectrometer and give 15 minutes to warm up per the manufacturer's instructions.

The spectrometer detected the particles wavelength for the highest absorbance of diclofenac medicine at 282nm. Usually, the UV absorbance spectrum of the diclofenac provide this information.

**Step 3: Blank Correction:**

Make a blank solution by adding only the diclofenac dilution solvent to it. This is used to account for any background absorbance that the solvent and cuvette may have contributed.

Set the spectrometer to 100% transmittance (zero absorbance) at the wavelength of the drug's greatest absorbance, then add the blank solution to the spectrometer cuvette.

**Step 4: Measurement of Absorbance:**

Place the unidentified samples and each of the prepared standard solutions in their respective cuvettes.

Using a UV spectrometer, determine each solution's absorbance at the wavelength at 282 nm where the diclofenac absorbs the lightest.

**Step 5: Calibration Curve plotting:** Plot a graph for the standard solutions showing concentration (x-axis) versus absorbance (y-axis). In the event that measured each concentration more than once, use the average absorbance value.

Through the data points, trace the line or curve with the best fit. A linear fit is frequently appropriate for a calibration curve, but if the relationship is not linear, you should think about using a different curve-fitting technique (such as quadratic, logarithmic, etc.).

**Step 6: Quantification of Unknown Sample:**

Using a UV spectrometer, determine the absorbance of unidentified samples at the wavelength where the drug absorbs the lightest.

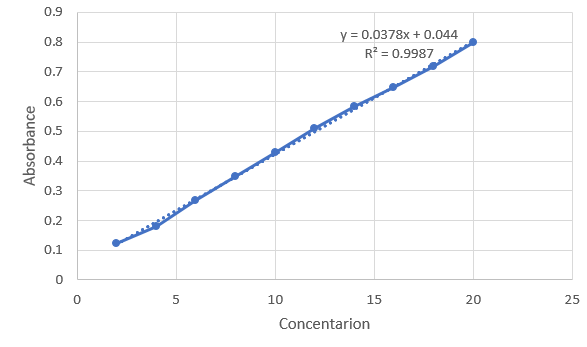
Based on the results of the unknown sample’s absorbance, use the calibration curve equation to calculate the drug's concentration there.

When working with medications and UV spectrometers, keep in mind to handle all solutions with caution, make sure to clean cuvettes properly, and adhere to safety measures. Additionally, to ensure accuracy and dependability, validate your calibration curve by examining samples with known concentrations**.**

# 8. **Observation table and statistical analysis:**

**Table 1: Calibration Curve data**

|  |  |
| --- | --- |
| concentration (μg/ml) | Absorbance(nm) |
| 2 | 0.124 |
| 4 | 0.181 |
| 6 | 0.268 |
| 8 | 0.347 |
| 10 | 0.429 |
| 12 | 0.511 |
| 14 | 0.582 |
| 16 | 0.646 |
| 18 | 0.717 |
| 20 | 0.797 |
| 100 | 2.59 |

****

**Figure 3 Calibration curve for Diclofenac sodium by UV spectroscopy**

# Comparative study of different bioavailability assessment:

Table 2 Table 2 Dissolution data for branded and generic tablets

|  |  |  |
| --- | --- | --- |
| **Time (min)** | **Cipzen-D®(Branded)** | **Voltaren®(Generic)** |
| 10 | 36% | 32% |
| 20 | 53% | 47% |
| 30 | 71% | 64% |
| 40 | 75% | 67% |
| 50 | 83% | 75% |
| 60 | 86% | 76% |

**Fig 4 Comparative graph of Branded and generic tablet for dissolution studies**

# 10. Result and discussion

This finding is favourable as it shows that generic product has similar *in vitro* drug release characteristics to the branded product. In such a case, it may be reasonable to assume that generally product will also have pharmaceutics and bioavailability *in vitro*. The dissolution profiles of generic and branded diclofenac sodium tablets were similar, suggesting that both formulations release the drug at the compatible rate. So, it is misconception that only expensive/ branded products(medicine) are good and effective.