EVALUATION OF THE BIOAVAILABILITY OF DICLOFENAC DERMAL PRODUCTS

Short title: PK and TS of diclofenac dermal products

UMB IRB #: HP-00067047

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Sponsor:

Food and Drug Administration
Office of Generic Drugs

Grant Number: 1U01FD004947

NCT03145259

Version 11.0

07 June 2019

STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) and the applicable Food and Drug Administration and other Department of Health and Human Services regulatory requirements.

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

PROTOCOL SUMMARY

Title:	Evaluation of the bioavailability of diclofenac dermal products
Population:	Healthy adults age 18 - 45 years
Number of Sites:	Single site: University of Maryland School of Medicine
Study Duration:	Approximately up to 1 year
Subject Participation Duration:	Approximately 10 weeks including the screening period
Description of Study Product:	Diclofenac epolamine (Flector®) patch 1.3%, Teikoku Seiyaku Co., Ltd; Diclofenac sodium (Pennsaid®) topical solution 2%, Horizon Pharma USA Inc.
Objective:	The present study aims to generate human tape stripping (TS) and pharmacokinetic (PK) data in healthy subjects for the purpose of establishing a reference for in vitro-in vivo correlation (IVIVC) with an in vitro model following the application of diclofenac products: Flector® (diclofenac epolamine 1.3% patch) or Pennsaid® (diclofenac sodium 2% topical solution).
Description of Study Design:	The study will be an open-label, non-placebo controlled, crossover study (n=12 healthy subjects) over 10 weeks includes three study sessions and up to a 45 day screening period with one week washout period between study sessions. The study contains nine procedure days: Study Session 1 • **Procedure Day 1:** Flector® patches (4 patches) containing 1.3% diclofenac epolamine to be worn for 10 h. Blood samples obtained on **Procedure Days 1, 2** and **3**. **Study Session 2** • **Procedure Day 4:** Pennsaid® topical solution (2 g of solution applied to 200 cm^2 area on each upper arm for a total of 4 g) containing diclofenac sodium to be worn for 6 h. Blood samples obtained on **Procedure Days 4, 5** and **6**. **Study Session 3** • **Procedure Day 7:** Six applications of Flector® 1.3% patch pieces and six applications of Pennsaid® topical solution 2%;

removal of patches at 10 h and solution at 6 h, followed by tape stripping at 10 h and 6 h, respectively (Uptake or Absorption)

- **Procedure Day 8:** Tape stripping at 27 or 23 h [17 h after removal] (Clearance)
- **Procedure Day 9:** Tape stripping at 51 or 47 h [41 h after removal] (Clearance)

A) Pharmacokinetics

Each subject will be his/her own control (pre-dose blood sample) and each subject will sign an institutional review board–approved consent form explaining the purpose, nature, risks, benefits, and duration of the study. The study will be conducted in accordance with good clinical practice guidelines and with the ethical principles originating in the Declaration of Helsinki.

The subject's skin in the area of application will be relatively free of hair before patch and topical solution application. For each treatment, the patch/solution will be applied to a previously unused application site. Blood samples (approximately 5 mL (1 tsp) each) will be drawn in BD vacutainer tubes. On Procedure Days 1, 2, 3, 4, 5 and 6, blood samples will be obtained as follows:

- Within 60 min pre-patch/solution application and then up to 51 h for patches and 47 h for solution, during wear and post patch/solution removal. No blood samples will be obtained during Procedure Days 7, 8 and 9.

B) Residual Drug Analysis of Diclofenac Patches

In conjunction with the above described study residual drug analysis will also be conducted for the previously worn Flector® patches and patch pieces from Study Session 1-3.

- Prior to administration to the subject as described in Part A, the patches will be weighed and the weight recorded.
- The pouch, release liner and all items coming into contact with the patches (gloves, forceps, etc.) to be applied in Part A will be retained for analysis.
- The used patches will be retained for drug content analysis.

- Upon removal of the patches after appropriate wear time, the skin (at site of application) will be swabbed and the swab retained for drug content analysis.

- All items coming into contact with the patches during removal from the subject will be stored in a separate labeled sealable pouch for analytical retention and drug content analysis.

C) Tape Stripping (TS) of diclofenac patch and topical solution (Study Session 3)

- Diclofenac products (6 sites/product, 3 sites/arm/product) will be applied to each arm. All of the patches will be removed at 10 h and solution at 6 h. One-third of the patch sites at 10 h and one-third of the solution sites at 6 h will be tape stripped (uptake or absorption), one-third of the patch sites at 27 h and one-third of the solution sites at 23 h will be tape stripped (clearance) and remaining patch sites at 51 h and solution at 47 h will be tape stripped (clearance).

- Tape strips applied and removed after patch/solution removal are extracted to determine drug concentration in stratum corneum, which is the outer layer of the epidermis.

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The purpose of this project is to investigate the development of appropriate methods to determine the in vitro/in vivo correlation (IVIVC) of drug absorption from dermal products, which has represented a longstanding challenge. The model drug for this study is diclofenac, which is available in two different formulations, patch and topical solution, approved by the United States FDA. This healthy human subject study will provide the carefully designed protocols and data for direct comparison (IVIVC) with an in vitro human skin diffusion study model. Generic drugs are approved based upon bioequivalence (BE) testing, and with respect to oral drug delivery, the accepted BE approach is relatively straightforward and is principally based on matching blood level profiles. In some cases, in vitro dissolution tests can be done to help determine the bioequivalence of an oral dosage form. For topical drug products in the United States, with the exception of corticosteroids and a few other products, a comparative clinical endpoint study is often necessary for approval of a generic product or for replacement of an approved dermatological drug product with one that has major changes. Comparative clinical trials are relatively insensitive, time-consuming and costly. It is especially difficult to gain the adequate statistical power needed to evaluate bioequivalence in some dermatological conditions, and can require a large number (i.e., hundreds[1,2]) of subjects.

This study supports FDA's continuing effort to identify the most accurate, sensitive, reproducible and efficient methods to evaluate topical dermatological drug products.

In 1998, the FDA issued draft guidance for the dermatopharmacokinetics (DPK) method to assess the bioequivalence of topical products for application to the skin[3]. The approach required quantification of the total amount of drug in the stratum corneum (SC) as a function of time, akin to the typical concentration-time profiles of traditional PK studies using blood sampling. Tape-stripping, a technique that involves multiple applications and removal of individual pieces of tape to a formulation-treated area of the skin of a healthy human subject, is the biosampling method used in TS to remove layers of the SC in serial. The draft guidance was withdrawn in 2002 following the inconsistent results of two studies[4]. The lack of agreement in the results prompted concern about both the reproducibility and the adequacy of this method to assess drugs whose target site is beyond the SC.

Considerable subsequent effort was directed at identification of problems/limitations of the original TS protocol and development of an improved procedure to generate reproducible data of much higher quality[5,6].

The end product of this research was a relatively straightforward method that examines only one uptake and one elimination time per formulation. This allows replicate sites to

be studied, and higher statistical power to be achieved with fewer volunteers. No tapes are discarded, but tapes are combined and extracted in groups to achieve high concentrations for analysis. The total amount of drug in the SC is measured for each site, and the ratios of these quantities for a reference listed drug (RLD) and test formulations can then be compared in the usual "80:125" area under the curve bioequivalence fashion. The method has been successively applied retrospectively to the comparison of retinoid gels and prospectively to the analysis of DPK data obtained for 3 econazole creams.

It follows that TS data obtained with the optimized protocol, which emerged from a previously FDA-funded project, represent suitable in vivo information for comparison and correlation with in vitro skin penetration. This study will evaluate two commercially available products; Flector® (diclofenac 1.3% patch) and Pennsaid® 2% diclofenac topical solution when administered in healthy adults. One uptake/absorption TS time point sample will be obtained, and two clearance time points will be obtained. Important systemic pharmacokinetic data in the form of blood drug levels will also be gathered during an identical protocol of absorption and clearance phase, in order to be able to cross compare all three methods, in vivo systemic PK, in vivo TS, and in vitro permeation techniques for thorough IVIVC. The diclofenac patch and topical solution have been chosen for this study because they are one of the few drug product types that will provide detectable blood levels of drug. Upon completion of this in vivo study, skin and blood diclofenac levels obtained after application of each product will be coupled with in vitro data generated in our laboratory at UMSOP to develop IVIVC models. These models would help in predicting in vivo skin and blood diclofenac levels from in vitro studies. The measurement of blood drug levels is the gold standard test for evaluation of pharmacokinetic parameters for systemically acting products. The systemic blood levels measured in this study will allow us to evaluate the TS and in vitro studies, as these latter two methods are currently not accepted as regulatory standard of practice. Some topical products may not provide measurable systemic blood levels, so in that case, future surrogate methods for clinical endpoint studies may be using TS or in vitro studies. The diclofenac product has systemic absorption to allow us to validate the two non-standard techniques.

2.2 Rationale

The goal of this study is to help establish more efficient pathways for approval of topical drug products by developing better tools to assess bioavailability. This study with Flector® (diclofenac epolamine 1.3%) topical patch and Pennsaid® (diclofenac sodium 2%) topical solution will provide sufficient systemic concentrations of diclofenac to characterize the systemic bioavailability. The systemic concentration will be compared with the local bioavailability of diclofenac in the skin using the products, measured by TS the skin to quantify the concentrations of diclofenac in the SC. The same products will also be studied (under a parallel but separate in vitro study protocol) using an in vitro permeation test (IVPT) model with excised human skin which has demonstrated IVIVC in previous

studies. This research will help establish whether the IVPT model correlates with and is predictive of in vivo bioavailability, and whether IVPT and/or in vivo TS can be utilized as part of a collective weight-of-evidence approach to support an approval pathway for topical drug products.

The multi-dimensional design of the current study minimizes the human subject research that would otherwise be expanded by the performance of multiple independent studies to assess local and systemic bioavailability, all of which can be now be directly compared in this study to support multiple, inter-related in vivo analyses relating to the bioavailability and bioequivalence. These coordinated evaluations also collectively provide the in vivo reference datasets that are needed to establish IVIVC for the IVPT model which may then be utilized to support a new pathway for approval of numerous topical drug products for patients.

Flector® patch and Pennsaid® topical solution

	Flector® 1.3% patch	**Pennsaid® 2% topical solution**
Inactive ingredients	1,3-butylene glycol, dihydroxyaluminum aminoacetate, disodium edetate, D-sorbitol, fragrance (Dalin PH), gelatin, kaolin, methylparaben, polysorbate 80, povidone, propylene glycol, propylparaben, sodium carboxymethylcellulose, sodium polyacrylate, tartaric acid, titanium dioxide and purified water	dimethyl sulfoxide USP (DMSO, 45.5% w/w), ethanol, purified water, propylene glycol and hydroxypropyl cellulose
Formulation	patch	topical solution
Manufacturer	Teikoku Seiyaku Co., Ltd	Horizon Pharma USA Inc.

3 OBJECTIVES

3.1 Study Objectives

1) Generate human PK data for the purpose of establishing an IVIVC model by collecting data following the application of two diclofenac formulations: Flector® 1.3% diclofenac epolamine patches and Pennsaid® 2% diclofenac sodium topical solution.

2) Generate TS data for the purpose of establishing a fundamental IVIVC model by collecting skin concentration data following the application of two diclofenac formulations: Flector® 1.3% diclofenac epolamine patches and Pennsaid® 2% diclofenac sodium topical solution.

3.2 Study Outcome Measures

For the TS study the main outcome measure is the ratio of clearance and uptake of diclofenac drug concentrations for each diclofenac formulation. For the PK study the main outcome measure is the maximum serum concentration (C_{max}); time of maximum serum concentration (T_{max}) of diclofenac and area under the curve (AUC) attained from Flector® 1.3% diclofenac epolamine patches and Pennsaid® 2% diclofenac sodium topical solution. In addition, the residual drug content from worn Flector® patches will be determined in order to attempt to estimate total amount of absorbed diclofenac.

4 STUDY DESIGN

This is designed as an open-label, non-placebo controlled, crossover study. The products tested are already FDA approved; however, the products are used outside of the approved indications by using healthy subjects. The products being tested are for research purposes only; this is not a treatment study. The study is not blinded because PK and TS assessment is not subject to participant and/or observer bias. The study will consist of three study sessions. Each of the 12 selected subjects will be enrolled to complete three study sessions, nine procedure days. There will be no overnight stays during the study. There will be at least a one week washout period between each study session.

The studies are as follows:

STUDY SESSION 1 (PK study)
Procedure Day 1: Four Flector® patches [140 cm^2 each; two patches/arm] will be applied for 10 h and removed. Blood samples will be collected at specified times.
Procedure Day 2: Blood samples will be collected at specified times.
Procedure Day 3: Blood samples will be collected at specified times. There will be at least a one week washout period before proceeding to the next study session.

STUDY SESSION 2 (PK study)
Procedure Day 4: Pennsaid® topical solution [4 g of solution over 400 cm^2 area; 2 g/200 cm^2 on each upper arm] will be applied to the skin for 6 h and removed. Blood samples will be collected at specified times.
Procedure Day 5: Blood samples will be collected at specified times.
Procedure Day 6: Blood samples will be collected at specified times. There will be at least a one week washout period before proceeding to the next study session.

STUDY SESSION 3 (TS study)
Procedure Day 7: Six sites (3 sites/arm/product) will be designated on the volar forearms for each diclofenac product and 1 site for a negative control (volar skin area where no drug is applied). Flector® 1.3% patches [8.25 cm^2 each] and Pennsaid® 2% topical solution [82.5 mg/8.25 cm^2 each] will be applied. At 10 h post application for patches and 6 h post application for solution, all products will be removed from their respective sites. Two sites for each product will be tape stripped at the designated uptake (absorption) time (10 h and 6 h post application).
Procedure Day 8: An additional two sites for each product will be tape stripped at the designated clearance period (27 and 23 h post application; 17 h after product removal).
Procedure Day 9: The remaining two sites for each product will be tape stripped at the designated clearance period (51 and 47 h post application; 41 h after product removal).

5 STUDY ENROLLMENT AND WITHDRAWAL

Only healthy adult volunteers who meet the inclusion/exclusion criteria will be eligible for enrollment into this study. Twelve subjects will be recruited as well as at least another five alternates who could replace subjects who drop out from the study for any unforeseen reason. The study population selected for this study includes healthy adult men and women without other comorbidities ages 18-45, inclusive. The selection criteria are designed to exclude people who might have medical conditions that could pose a safety risk and people whose medical conditions might interfere with the objectives and results of the study.

Subjects will be recruited by local advertisements to the study center, online recruitment, newspaper ads or The Elm Weekly. Potential subjects who are interested in the study will be informed of the study and if they wish to participate, will receive additional study information, including an onsite screening appointment and informed consent form. Each of the 12 selected subjects enrolled will be expected to complete all three study sessions.

We aim to target at least 40% participants of each gender. There are no specific ethnicity/race category recruitment targets, although such information will be recorded by the researchers.

5.1 Subject Inclusion Criteria

Subjects are eligible for this study if they fulfill the inclusion criteria specified below:

1. Men or non-pregnant, women who are of any ethnic background between the age of 18 and 45 years old.

2. Subjects must be non-smokers (must have refrained from the use of nicotine-containing substances, including tobacco products (e.g., cigarettes, cigars, chewing tobacco, snuff, gum, patches or electronic cigarettes) over the previous 2 months and are not currently using tobacco products.

3. Provide written informed consent before initiation of any of the study procedures.

4. Agree not to participate in another clinical trial/study during the study period or to participate in an investigational drug study for at least 1 month after the last study session.

5. Able to adhere to the study protocol schedule and study restrictions.

6. Able to participate in all study sessions.

7. Has a volar forearm of either at least 24 cm (9.45 inches) in length or of sufficient size that can accommodate the products to be tested in a study area that begins at least 5 cm (1.97 inches) above the wrist and ends a minimum of 0.5 cm (0.197 inches) below the antecubital fossa (i.e., the bend in the arm at the elbow).

8. Subjects have upper arms large enough to allow for the placement of two 140 cm^2 [21.7 in^2] patches (distance from acromion process of the scapula to olecranon process should be a minimum of 35 cm [13.8 inches]; circumference of upper arms should be a minimum of 28 cm [11.02 inches] and 200 cm^2 [31 in^2] area for application of solution.

9. Subjects deemed to be healthy as judged by the Medically Accountable Investigator (MAI) and determined by medical history, physical examination and medication history.

10. Negative urine drug screening test (cannabinoids, amphetamines, barbiturates, benzodiazepine, cocaine, methadone, opiates, PCP).

11. Have normal screening laboratories for white blood cells (WBC), hemoglobin (Hgb), platelets, sodium, potassium, chloride, bicarbonate, blood urea nitrogent (BUN), creatinine, alanine transaminase (ALT) and aspartate aminotransferase (AST).

12. Have normal screening laboratories for urine protein and urine glucose.

13. Female subjects must be of non-childbearing potential (as defined as surgically sterile [i.e. history of hysterectomy or tubal ligation] or postmenopausal for more than 1 year), or if of childbearing potential must be non-pregnant at the time of enrollment and on the morning of each study day, and must agree to use hormonal or barrier birth control such as implants, injectables, combined oral contraceptives, some intrauterine devices (IUDs), sexual abstinence, or a vasectomized partner.

14. Agrees not to donate blood to a blood bank throughout participation in the study and at least 3 months after the last study session.

15. Have a normal ECG; must not have the following to be acceptable: pathologic Q wave abnormalities, significant ST–T wave changes, left ventricular hypertrophy, right bundle branch block, left bundle branch block. (sinus rhythm is between 55–100 beats per minute).

16. Have normal vital signs (*see Appendix C*)

    - Temperature 35-37.9°C (95-100.3°F)
    - Systolic blood pressure 90-165 mmHg
    - Diastolic blood pressure 60-100 mmHg
    - Heart rate 55-100 beats per minute
    - Respiration rate 12-20 breaths per minute

5.2 Subject Exclusion Criteria

Subjects will be excluded for any of the following conditions/reasons:

1. Women who are pregnant, lactating, breast feeding or have a positive serum pregnancy test at enrollment or positive urine pregnancy test on the morning of the first day of each study session.
2. Smokers (current use or use over the previous 2 months of nicotine-containing substances, including tobacco products (e.g., cigarettes, cigars, chewing tobacco, snuff, gum, patch or electronic cigarettes).
3. Participation in any ongoing investigational drug trial/study or clinical drug trial/study.
4. History as either reported by the subject or evident to the Medically Accountable Investigator (MAI) of infectious disease or skin infection or of chronic skin disease (e.g., psoriasis, atopic dermatitis).
5. History of diabetes.
6. History of significant skin cancers (e.g., melanoma, squamous cell carcinoma), except basal cell carcinomas that were superficial and did not involve the investigative sites.
7. Body Mass Index (BMI) ≥30 kg/m^2.
8. History of chronic obstructive pulmonary disease or cor pulmonale, or substantially decreased respiratory reserve, hypoxia, hypercapnia or pre-existing respiratory depression.
9. Active positive Hepatitis B, C and/or HIV serologies (see *Appendix B*).
10. Positive urine drug screening test.
11. Use of any prescription medication during the period 0 to 30 days or over-the-counter medication e.g. antihistamines, topical corticosteroids during the period 0-5 days before entry to the study (vitamins, herbal supplements and birth control medications not included).
12. Currently taking daily oral nonsteroidal anti-inflammatory drug [NSAIDs] (aspirin, ibuprofen, naproxen, etc…).
13. Currently taking daily anticoagulants or within the past month prior to entry into the study (warfarin, heparin, rivaroxaban, dabigatran, etc…) ACE-inhibitors, cyclosporine, diuretics, lithium or methotrexate.
14. Donation or loss of greater than one pint of blood within 60 days of entry to the study.

15. Any prior adverse reaction or hypersensitivity to diclofenac, aspirin, ibuprofen, naproxen or other nonsteroidal anti-inflammatory drug (NSAID), other inactive ingredients in the patch or topical solution or to adhesives or tapes used to cover or tape strip the treatment sites.

16. Received an experimental agent (vaccine, drug, biologic, device, blood product or medication) within 1 month before enrollment in this study or expects to receive an experimental agent during the study.

17. Eat or drink anything containing alcohol within 24 hours prior to dose administration.

18. Any condition that would, in the opinion of the Medically Accountable Investigator (MAI), place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

19. Subject has an obvious difference in skin color between arms or the presence of a skin condition, excessive hair at the application site (upper arms/volar forearms), sunburn, raised moles and scars, open sores at application site (upper arms/volar forearms), scar tissue, tattoo, or coloration that would interfere with placement of diclofenac products, skin assessment, or reactions to diclofenac.

20. History of asthma or urticaria, hypertension, myocardial infarction, thrombotic events, stroke, congestive heart failure, impaired renal function or liver disease.

21. History of gastrointestinal bleeding or peptic ulcer disease.

6 STUDY PRODUCT

6.1 Study Product Description

6.1.1 Flector® (diclofenac epolamine 1.3%) patch [140 cm^2]

Flector® is a prescription diclofenac-containing patch that releases diclofenac through the skin into the body. The patch releases diclofenac over a 12 h period, the recommended duration of patch application. The patch is for the topical treatment of acute pain due to minor strains, sprains and contusions.

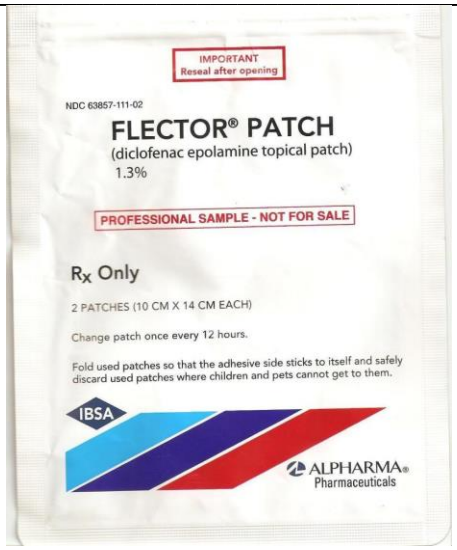
6.1.2 Pennsaid® (diclofenac sodium 2%) topical solution

Pennsaid® 2% topical solution is a prescription diclofenac-containing solution that releases diclofenac through the skin into the body. The solution releases diclofenac over a 12 h period. The solution is a nonsteroidal anti-inflammatory drug (NSAID) indicated for the treatment of the pain from osteoarthritis of the knee(s).

6.2 Formulation, Packaging, and Labeling

6.2.1 Flector® (1.3% diclofenac epolamine) patch

Active ingredient (in each patch): diclofenac epolamine, 180 mg (13 mg per gram adhesive) in an aqueous base. In addition to the active ingredient (diclofenac epolamine), the following inactive ingredients are present in the patch: dihydroxyaluminum aminoacetate, disodium edetate, D-sorbitol, fragrance (Dalin PH), gelatin, kaolin, methylparaben, polysorbate 80, povidone, propylene glycol, propylparaben, sodium carboxymethylcellulose, sodium polyacrylate, tartaric acid, titanium dioxide and purified water. This patch is manufactured by Teikoku Seiyaku Co., Ltd. This product should be stored at room temperature.



6.2.2 Pennsaid® 2% diclofenac sodium topical solution

In each pump of solution there is 1 g of solution (20 mg diclofenac sodium per gram solution). In addition to the active ingredient (diclofenac sodium), the following inactive ingredients are present in the solution: dimethyl sulfoxide USP (DMSO, 45.5% w/w), ethanol, purified water, propylene glycol and hydroxypropyl cellulose. This solution is manufactured by Horizon Pharma USA Inc. This product should be stored at room temperature.

7 PHARMACOKINETICS AND STATISTICAL CONSIDERATIONS

7.1 Study Hypothesis

TS and PK

The tested diclofenac products are non-bioequivalent hence, we will test the null hypothesis (H_0) that the mean clearance/uptake diclofenac amount ratio, and the PK parameters (C_{max} and AUC) between these products are different (i.e., outside the 80–125% range).

7.2 Analyses

Diclofenac concentrations will be measured in serum samples collected from each subject. Blood samples (approximately 5 mL (1 tsp)) will be collected during Study Session 1 prior to dosing and then at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 24 h, 27 h, 30 h, 32 h and 51 h post-patch application. Blood samples (approximately 5 mL (1 tsp)) will be collected during Study Session 2 prior to dosing and then at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 7 h, 23 h, 26 h, 29 h, 31 h and 47 h post-solution application. After the PK study, TS study will be conducted at 10 h [patches] and 6 h [solution] (skin drug uptake/absorption), 27 h [patches] and 23 h [solution] (skin drug clearance), and 51 h [patches] and 47 h [solution] (skin drug clearance) post product application. Non compartmental analyses (NCA) will be conducted to estimate the PK parameters such as: maximum serum concentration (C_{max}); apparent elimination rate constant (k); apparent half-life ($t_{1/2}$), calculated as 0.693/k; $AUC_{0\text{-last}}$ of the serum concentration–time determined by the linear trapezoidal method; and AUC value extrapolated to infinity (AUC_{inf}), calculated as the sum of $AUC_{0\text{-last}}$ and the area extrapolated to infinity: $AUC_{inf} = AUC_{0\text{-last}} + C_{last}/k$ where C_{last} would be the last quantifiable concentration. All NCA analyses will be conducted using Phoenix® WinNonlin® 6.4 (Pharsight, a Certara Company, CA).

7.3 Final Analysis Plan

An objective of this study is to determine PK parameters (C_{max}, AUC and ratio of SC drug concentration during uptake and clearance) of diclofenac in healthy adults and TS data after using Flector® (diclofenac epolamine 1.3% patch) and Pennsaid® (2% diclofenac sodium topical solution) for the purpose of IVIVC by collecting data over two separate application periods.

Analysis of variance (ANOVA) followed by post-hoc Bonferroni test will be used for comparing the differences in the means of the PK parameters and significant differences will be declared at $p<0.05$. The statistical comparisons will be conducted as follow:

If diclofenac PK concentrations are found to be non-normally distributed, then we will examine Box-Cox transformations (e.g., log, square-root, etc.) that can achieve normality. If no transformation can achieve normality, then will use permutation tests to compute empirical *p*-values, and will use the bootstrap to compute standard errors and confidence intervals that account for within-person correlation.

IVIVC will be conducted comparing PK parameters and profiles to predicted PK parameters and profiles using IVPT and in vitro TS results. Multiple methods will be implemented to develop an IVIVC. The first method is to compare the steady state concentrations. The predicted steady state concentration using IVPT data will employ the following formula:

$$C_{ss} = \frac{J_{ss} * A}{CL}$$

The second method will compare the PK profiles of the clinical and IVPT study by predicting diclofenac concentrations at each time point in the IVPT study and comparing it to the clinical PK profile. The third method will be to determine and compare residual patch analysis between in vitro and in vivo.

In terms of developing an IVIVC for the TS study a comparison will be made between in vitro and in vivo uptake (absorption) and clearance diclofenac amounts. The second method is to compare the clearance to uptake diclofenac amount ratio between in vitro and in vivo.