International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Thematic Special Issue 2022

Review Article

PHYSIOCHEMICAL, *IN-VITRO, EX-VIVO* AND *IN-VIVO* EVALUATION OF TRANSDERMAL PATCHES BASED ON QBD APPROACH: AN OVERVIEW

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Received: 15 Apr 2021, Revised and Accepted: 30 May 2022

ABSTRACT

There are various forms of prescription drugs available in the market for human and animal purposes, which may be administered for acute or chronic treatment. And a variety of dosage regimens of different dosage forms are found in general, among which one of the treatment modes is the transdermal route. The Transdermal Tract Route is a more accurate way to get into the skin without pain, faster to systemic circulation than the oral route and the drug reaches the systemic circulation in a controlled transmission.

Improving the transdermal system poses an additional challenge to maintain Quality by Design (QbD) with quality, consistency, reproduction, stability and efficiency. With the QbD method of pharmaceutical product production, product quality is considered much faster than waiting until the end to be tested for quality. In essence, this includes determining the sources of the unpredictability that may be contributing to the process. This approach ensures that the causes of quality problems are identified. The main result is a pharmaceutical product manufactured to meet pre-determined quality requirements from the outset.

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Critical quality attribute (CQA) and Critical Process Parameters can be used to establish QbD (CPP). During the construction of a transdermal design, it is important to check the various key parameters that help maintain the Quality Target Product Profile (QTPP) of the drug product.

Keywords: Transdermal patch, Physical evaluation, Physicochemical property, *In vitro* transdermal, *Ex vivo* transdermal, *In vivo* transdermal

 DOI: https://dx.doi.org/10.22159/ijap.2022.v14ti.20 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

The regulatory body guide addresses new applications for marketing authorization (including standard or abbreviated applications) and the subsequent distribution of transdermal patches for systematic submissions [1]. Guidance is provided on descriptive quality requirements, development (Drug product character status), production and auxiliary features of excipients, drug product control, packaging and stability of transdermal packs. In particular, *in vitro* performance tests regarding drug release, adhesion and skin penetration are discussed, as well as the relationship with clinical and *in vivo* performance.

All regulatory bodies state that CQAs operate in any "physical, chemical, biological, or microbiological or biological areas" that must be within the limits or scope to ensure that a pharmaceutical product meets the required quality. The identification of CQAs usually occurs during the initial phase of process validation. This is the stage of designing a process. This is a good time to establish acceptable limits and scope and to establish agreements for measurement, data collection, and data analysis. CPP is a variable that can affect CQA. Therefore, CPPs should be monitored to enable early and accurate detection of deviations without acceptable limitations that will affect product quality. Of course, not all process parameters are the same as some will have a greater impact on CQAs than others. In order to get a good result, it is important to prioritize CPPs over other process parameters as they will be large. In all process parameters, CPPs should be strictly controlled. The measurement system to estimate the risk parameter is listed in table 1.

Table 1: Overview of relative ranking system in order to estimate risk parameter:

Low	Broadly acceptable risk. No further investigation is needed.
Moderate	Risk is acceptable. Further investigation may be needed in order to reduce the risk.
High	Risk is unacceptable. Further investigation is needed to reduce the risk.

Risk factor of the Critical Quality Attribute for the transdermal patch dosage form would be classified based on the assessment as per the above-mentioned ranking system. Low-risk CQA would be justified with the narrated explanation, moderated risk CQA would be explained with quite sufficient elaboration and the high risk CQA's would be explained with more detail to understand more about the transdermal patch dosage forms.

Physiochemical evaluation of transdermal patch

The risk assessment of the Physiochemical parameter and justifications are listed in table 2.

Water vapour transmission rate

The flow of vapor in the membrane is measured by the infiltration of water vapor. Under test conditions, the rate of water vapor transfer in a unit area per unit of air pressure variation. It is known as the water vapor transfer rate (WVTR) or vapor transfer rate (MVTR). There are various test methods available to measure WVTR.

A. Method of Gravimetric
B. Method of Infrared sensor
C. Method of Electrolytic sensor
D. Method of Humidity sensor

Table 2: Risk assessment of the physiochemical parameter and justification

Transdermal patch CQA's	Risk assessment	Justification
Description	Low	Description is not only limited to appearance of transdermal patch, but also includes color, odor, size, etc. This CQA will not impact the quality of the transdermal patch. Thus this is not a critical parameter.
Surface morphology	Low	Surface morphology may vary based on the design requirement of the transdermal patch. Generally, morphology of the transdermal patch is expected to be smooth or as per the requirement of the market. This CQA will not impact the quality of the transdermal patch.
Thickness	Low	Variation in the Thickness leads to change in the drug content in transdermal patch. However this CQA can be controlled by proper batch setting during the manufacturing of the transdermal patch. This CQA will not impact the quality of the transdermal patch.
Tensile strength	High	Tensile strength is a measurement of the force required to pull transdermal patch to the point where it breaks. This CQA is considered to be high.
Dose uniformity	High	Improper mixing of drug substance throughout the transdermal patch will lead to poor drug distribution in the transdermal patch. So this is a critical CQA.
Moisture content	Low	Moisture content of the transdermal patch is determined by the composition and the process involved in manufacturing of transdermal patch. Hence this is not a critical CQA.
Shear Adhesion	High	This test establishes the cohesive strength of an adhesive polymer. This is one of the important CQA to ensure the quality of the transdermal patches.
Peel Adhesion	High	This CQA ensures the force required to remove an adhesive coating from a test substrate. This improper adhesion of the patches will impact the quality of the product. So this is a critical CQA.
In vitro drug release study	High	*In vitro* release testing determines the rate and degree of active ingredient release from a transdermal patch. Despite the fact that the test does not simulate *in vivo* performance, it is an important quality attribute to specify in the finished product release and shelf-life specification. As a result, this is a crucial CQA.

Method of gravimetric

The Transdermal Patch sample should be placed in a testing container containing water or desiccant inside. The test container should be placed in a controlled environment with equal temperature, humidity, and airflow. Water vapor enters the model and enters the dry side. The rate of transfer of water vapor and other factors can be determined by measuring the variation in the weight of the constant testing vessel [1]. An image of the Gravimetric WVRT tester is shown in fig. 1.

Fig. 1: Gravimetric WVTR test system (C360M)

Method of Infrared sensor

The Transdermal Patch test sample should be placed in a distribution cell, which is divided into a dry room and a controlled humidity chamber. The flow of dry nitrogen should sweep the dry side of the statue, and the vapor flowing from the sample from the controlled humidity chamber should be supplied with dry nitrogen to the infrared sensor, which can produce equal electrical signals. Electrical signals were analyzed and calculated to determine the rate of evaporation. Dry nitrogen flows between the sample of all package samples, while the outside of the sample is kept at a high humidity level [2]. The fig. of the infrared WVTR sensor tester is shown in fig. 2.

Fig. 2: Infrared sensor WVTR Test System (PERME®C390)

Method of electrolytic sensor

The continuous moisture difference between the two sides of the test sample is produced at a certain test temperature. The vapor passes through the specimen to the dry side before being transferred to the sensor, producing equal electrical impulses. The rate of evaporation and other parameters can be determined by analyzing and calculating these electrical signals [3]. An image of the Electrolytic sensor tester WVTR is shown in fig. 3.

Fig. 3: Electrolytic sensor WVTR Test System (PERME®TSY-W3)

Method of humidity sensor

Between the dry and moist chambers, place the pre-conditioned transdermal patch specimen. The dry chamber's humidity sensor measures variations in humidity and calculates the time it takes for humidity to rise from the lower preset value to the higher one. By measuring and analysing the water vapour transmission rate and the coefficient on a regular basis, the water vapour transmission rate and coefficient may be calculated [4]. The fig. of Humidity sensor WVTR tester is shown in fig. 4.

Fig. 4: Humidity sensor WVTR test system (PERME®W3/130)

Percentage elongation/Tensile strength

Mechanical properties such as the percentage of the leave break and the grip strength are determined by recognizing the length just before the break point, the elongation percentage can be determined by the formula mentioned below.

Elongation percentage = L1-L2/l2 × 100

There, L1 is the final length of each string

L2 is the first length of each line.

Percentage length and stiffness during breaks give an indication of the strength and elasticity of the film. It is suggested that films suitable for wound dressing should at best be strong and flexible. The structure is measured according to ASTM D638 using the Universal UK (UTM) testing machine (Model 4309, Instron) [23]. A minimum of six samples were tested for each composition at room temperature and the average value should be recorded. The fig. of Elongation tester is shown in fig. 5.

Fig. 5: Elongation tester (Instron-ASTM D638)

ASTM D638 is made using tensile strength in the sample model and measuring different template structures under pressure. It is done on an international test machine (also called a tensile test machine) at rigid speeds from 1 to 500 mm/min until the template fails (yield or breaks). The increase in the length of the gauge after the break is divided by the length of the first gauge. Excessive stretching indicates high swelling of the transdermal patch.

Dose uniformity

The portion of the well-measured pool is cut into small pieces and placed in a volume flask. The flask content is dissolved in a suitable solvent and sonicated to ensure the complete dissolution of the drug from the patch. After allowing the solution to settle for about an hour, the supernatant was purified to achieve the required concentration using a suitable solvent. The solution was filtered using a membrane filter and analyzed according to the appropriate analysis (UV or HPLC) [5]. The acceptance (AV) value-for-quantity test table is shown in table 3.

Moisture absorption

The weight film is stored in desiccators at room temperature for 24 h and removed with a relative humidity of 84% (full solution of potassium chloride) in the desiccators and films are exposed to it until a constant weight is obtained. The moisture absorption percentage is calculated as the difference between the final and initial weight by the initial weight [6].

% moisture absorption = [last weight-initial weight]/initial weight X100.

Shear adhesion

Shear adhesions produce resistance to adhesives that are sensitive to tangential stress and, therefore, matrix bonding [6].

Cutting methods measure the force required to pull a normal patch from a normally flat surface (adhesive plate) in a manner consistent with the attached area. This vertical test indirectly measures the force required to skip a patch on a sticky plate by determining the time required to remove the normal patch from the sticky plate under normal load. In test strength tests, the adhesive should fail together, leaving a sticky layer on the adhesive plate or supporting layer. The results can only be considered as a true measure of the internal adhesive strength if this mode of failure occurs. When the adhesion size becomes larger, there are more shear layers, and thus the matrix cohesion becomes weaker. The grip strength is therefore related to the thickness of the peg matrix by a clear relationship [7, 8].

Table 3: Acceptance value (AV) for dose uniformity test

Variable	Definition	Conditions	Value
L1	Maximum allowed acceptance value		15.0 unless otherwise specified
L2	Maximum allowed range for deviation of each dosage unit tested from the calculated value of M	On the low side, no dosage unit result can be less than [1–(0.01)(L2)]M, while on the high side, no dosage unit result can be greater than [1+(0.01) (L2)]M. (This is based on an L2 value of 25.0.)	25.0 unless otherwise specified

Peel adhesion

Peeling is one of the most important features of leaflets, as it tightens the grip of the page, making it more painful to remove. Page resistance should not be construed as an indicator of bond strength because this feature is not always compatible with internal adhesion. Prior to splitting, the patch matrix and support layer are expanded and bent, making splitting a complex process. Therefore, the force required to extract a sticky piece for decades is higher than that required for storage [9].

The various mechanisms of failure of patches from a solid surface can be interpreted as a measure of matrix coupling and adhesion of the supporting layer. Generally, when the patch is peeled off, it is expected to remove it clean from the adherent (Case I), leaving no visible residue [10]. A schematic diagram of a weak patch mode is shown in fig. 6.

There are four types of failure that can occur when a clip is removed from a holding person. The only valid type of patch is Case I (failure to adhere). If the adhesive-sensitive adhesive does not adhere firmly to the supporting layer, it may transfer to the adherent, leaving the support layer outside the matrix (case II). If the matrix has strong adhesion strength but insufficient bonding strength, Case III occurs. A mixture of adhesive and adhesive failure occurs in Case IV. The transition from one type of failure to another is influenced not only by the additives but also by the peel level [11].

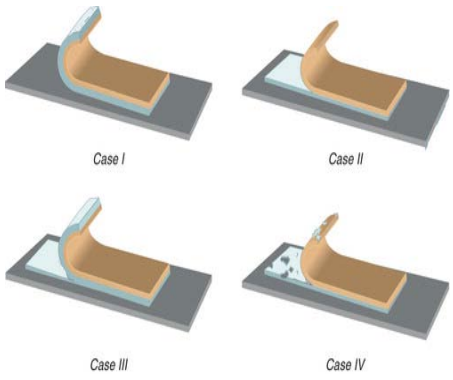

Fig. 6: Patch modes of failure

The method of determining adherence is determined by the type of pressure emitted during a particular activity. A detailed description of the recommended procedures for adhesive tape associations has already been published [10]. In short, standard test procedures require the use of a patch cord on a solid test plate (adhesive plate), usually made of stainless steel, as well as a pressure gauge to ensure touch; after a specified time, the strip is removed from the plate at a certain angle (180° or 90°) and speed (300 mm/min).

The friction is given to the supporting layer by using an adhesive matrix during the separation process. As a result, changes in behavior during separation may be due to structural changes, which influence flexibility and extension, as well as the size of the supporting layer. Indeed, as the thickness and/or stiffness of the supporting layer increases, so does the force required to reverse the supporting layer. At the same time, the line scanner creates a larger temporary arm, which leads to reduced page power and compensates for the lost power of the supporting layer. Because a lower amount of force is required to disable the supporting layer in the 90° page adhesion test rather than the 180° page adhesion test, the impact of the supporting layer structures is less noticeable. In addition, because the 180° page is a combination of solid and cutting processes, while the 90° page relies solely on rigid events, the average deviation of the data obtained by peel strength by 90° is lower than that obtained by page measurement power at 180° [12]. It is also noteworthy that, although both 90° and 180° page tests are drawn at the same rate, the front 90° test page doubles the 180° test rate. As a result, the adhesive effects of the two flat angles are not really comparable. For example, in the case of PSAs made of PSA neoprene rubber, 90 ° testing allowed the effect of PSA thickness to be fragmented, and page strength was about higher-order size than 180° test.

The thickness of the matrix affects the adhesion of the peach sheet in the same way as the grip of energy, but with a different one. When the peel strength is strong, the adhesive matrix becomes larger [13, 14], with a certain thickness [13, 14]. This feature can be explained by considering the effect of peel force of thickness. Dense PSAs do, in fact, increase the amount of flexible matrix, which is why it raises peel strength. The power of the peel falls through the thicker strips due to the increase of the angle and the temporal arm, which leads to a decrease in the separating function. The transformation of the PSA into a fracture area is large enough that a further increase in thickness is no longer reflected in a significant number of matrix thicknesses.

***In vitro* drug release study**

The *in vitro* release test determines the level and level of active ingredient release from the transdermal patch. Apart from the fact that testing does not mimic vivo performance, it is an important quality attribute that must be specified in the product release and the shelf life specification [15].

Dissolving tests or extraction tests using a suitable, non-abrasive membrane should be used, as shown in the European Pharmacopoeia (Ph. Eur.) Monograph for Transdermal Patches. Alternatively discriminatory methods higher than compensation methods may be used if necessary [15].

The transdermal patch should not be damaged or altered in any way during the testing or processing of the sample. Any sample preparation requirements should be discussed. If it is found that the modification of the sample does not affect the release/disposal of the drug, it may be possible to test only a sample of a specific area of the pool that is suitable for all intensities. If the patch is too large to fit into a standard exhaust test, or if sink conditions are not met by complete pads, the appropriateness of test samples can be considered from a volume measurement study of samples of various sizes [15].

The active ingredient from the *in vitro* drug release profile of a drug product should be noted and developed from clinical collections that have shown satisfactory efficacy. These should be used to support drug product specification in *in vitro* drug release/termination limits, ensuring that future product groups are of the same quality as key clinical collections [15].

Satisfactory evidence of discrimination must be provided in respect of:

1. Important production changes

2. Auxiliary materials and essential attributes of functional materials

3. Stability that indicates road strength

A summary of the development of the dispersion test should be provided, where the transdermal phase is tested under a variety of conditions (media, pH, apparatus, agitation, etc.). Testing conditions that provide the most appropriate discrimination should be selected. In the case of a low-volume media bath, the pH should be controlled during the completion test to avoid the effect of the dissolved active ingredient and/or auxiliary substances in the dispersion conditions during the test.

The pH of the middle area should be adjusted to pH 5-6, indicating the skin conditions of the body. For the same reason, the test temperature is usually set to 32 ° C (although the temperature may be higher if the skin is covered). 100 rpm is considered the standard deviation of Ph. Eur., Which also allows for aliquot pool phase testing. The latter may be the best way to achieve sink conditions, as long as cutting a piece of the pond is guaranteed so as not to interfere with the release process [16].

USP method 5, or paddle over disc is used for transdermal patch

In the Paddle-over-Disk method, the paddle and the merging vessel from Apparatus 2 and the addition of a stainless steel disk assembly designed to hold the transdermal system under the vessel. The sketch diagram of USP Apparatus 5 is shown in fig. 7 and 8.

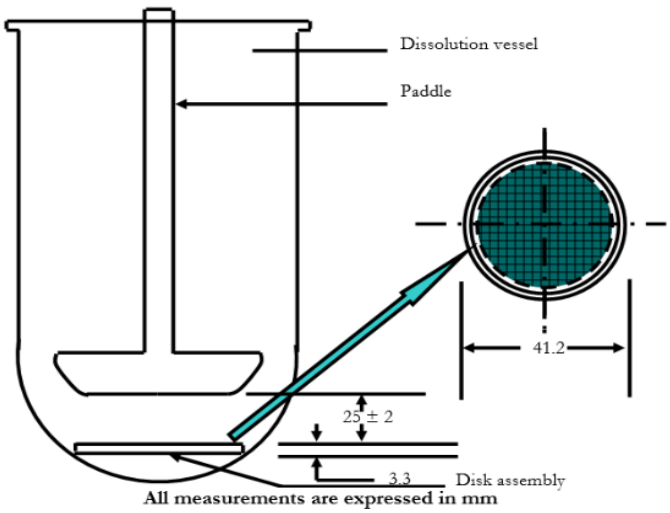

Fig. 7: USP apparatus 5

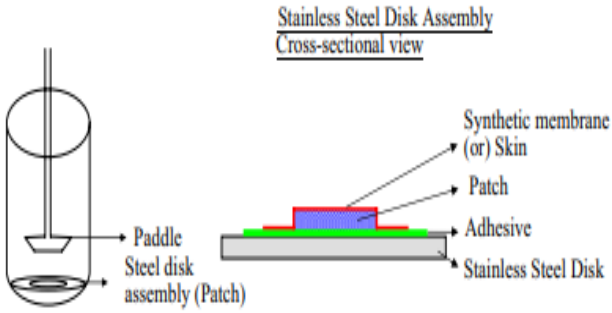

Fig. 8: Apparatus 5 with stainless steel disk assembly

The temperature is maintained at 32 °C±0.5 °C. The disk assembly holds the system flat and positioned so that the rental area is aligned with the bottom of the paddle blade. Apparatus is used to diagnose transdermal patches [17].

***In vitro* release testing by diffusion cell apparatus**

The purpose of the release test is to determine whether the product is suitable for release. Release testing methods are designed to ensure that a product meets production requirements rather than a specific product rating to determine its performance. Therefore, IVRT methods may not have as much bio relevance. The focus of the release trial is to ensure that the drug that spreads to the receptor media is the only thing that can be measured. Nothing in the media, membranes or any other part of the experimental approach should have an impact on the quality of distribution [16]. A picture of the robot distribution station is displayed on fig. 9.

Fig. 9: Phoenix RDS™ robotic diffusion station

- Receptor media is typically a mixture of organic and aquatic solution. In some cases, complete solvents should be used for melting problems. The media should maintain immersion conditions where the estimated 10x of the expected output should be dissolved in the media. The media should be selected by mass screening and by selecting the media that provides the best results [16].

- Membranes in an IVRT test are strictly to hold the dosage in place and separate it from the receptor media. This helps to keep the surface area constant and allows for a comparison of distribution levels relative to the location. Cells are not supposed to absorb the drug, or to extract anything from the receptor media that will stimulate or bias the analysis. Ideally, the membrane should not limit the amount of product distribution at all; however, a small amount of resistance is expected [18].

- Test length is usually 4 to 8 h with small sample 5 points. The length of the test and the sample points should be determined by the methodical process. Sampling should start after the membrane has had time to fully fill. In most cases 30 min to 1 hour is enough. Sampling must end before no more than 30% of the API is issued. Although in some cases no more than 10% of the output is better. This is due to the need for a continuous dose, where there is always a sufficient amount of the drug to drive the distribution process at high-speed conditions [18].

***Ex vivo* and *In vivo* evaluation of transdermal patches**

The risk assessment of the *Ex-Vivo* and *In vivo* Elevation of transdermal patches in table 4.

***Ex vivo* permeability**

Skin reactions are called percutaneous/dermal absorption:

• The reception of goods in a particular layer or structure, such as the penetration of a compound into the stratum corneum, is known as penetration [15]; and

• Permeation refers to the transfer of information from one layer to another which is functional and structurally different from the original [15].

To evaluate the penetration of active chemicals from the transdermal patch, *in vitro* permeation testing is commonly used during drug development. *In vitro* testing has the advantage of being able to control test conditions, so changes in an entry should only be made for changes in the transdermal patch and/or membranes used [15].

Although *in vitro* permeation testing is not possible to predict *in vivo* release, permeation profile visibility is considered an important indicator of product quality and performance, and may reflect thermodynamic activity of the active component in the product.

Table 4: Risk assessment of the *ex vivo* and *in vivo* parameter and justification

Transdermal patch CQA's	Risk assessment	Justification
Ex-Vivo Permeability	High	The evaluation of any dermal or transdermal medication delivery device must include a measurement of molecule percutaneous absorption. Understanding the parameters that determine optimal *in vivo* performance is a fundamental goal in the design and development of dermal or transdermal dose forms. Human studies provide the most trustworthy skin absorption data; nevertheless, such investigations are typically not practical during the early stages of developing a novel pharmacological dosage form or considering a new medication candidate. Thus, one of the main challenges of biopharmaceutical research is finding a correlation between *ex-vivo*, animal and human studies for the prediction of percutaneous absorption in humans. It is practically impossible to assess the skin permeability of materials using *in vivo* experiments alone. Consequently, numerous *ex-vivo* and *in vitro* models are frequently employed to assess drug skin permeation profiles and kinetic parameters. Hence, a method that can consistently correlate *ex-vivo* and *in vitro* data to shorten and economize the process of drug development and minimize the number of human studies is critically needed [19].
Skin irritancy	High	This method provides information on health hazard likely to arise from exposure to test substances via intradermal injection and/or epidemal application [27]. Test No. 406 OECD Guideline for testing of chemicals to be followed for this test. This test guideline is intended primarily for use with guinea pig, but recently mouse models for assessing sensitization potential have been developed [21]. This is a critical CQA.
Studies of cutaneous retention	High	Direct assessments of drug concentration in the whole skin and previously isolated skin layers, as well as the use of reconstructed skin models, can be used to assess cutaneous retention of medicines and other bioactive chemicals. Several procedures can be used to isolate the skin layers [21]. For the transdermal patch, this is a critical CQA.
In vivo evaluation of the skin permeation of drugs	High	Pharmacokinetic studies or cutaneous microdialysis are both used to assess *in vivo* skin absorption. These tests were carried out with human volunteers or animal models and protocols that had previously been authorized by an ethical council in both circumstances [21]. This is a critical CQA for the transdermal patch.
Preclinical pharmacokinetic evaluations	High	Clinical trials using human participants are preceded by preclinical pharmacokinetic analyses in animal models. The goal of these studies is to see if the drug's target plasma concentrations are reached or maintained for long periods of time after delivery of the transdermal formulation [21]. This is a critical CQA for the transdermal patch.
Cutaneous microdialysis	High	*In vivo* evaluations using cutaneous microdialysis have been used for quantification of cerebral neurotransmitters, glucose monitoring in diabetes mellitus, measurement of antibiotics levels, and measurement of antineoplastic drug levels in the target tissue [21]. The assessment of *in vivo* skin absorption of medications from topical and transdermal preparations is one of these applications. Traditional pharmacokinetic analyses look at the overall amount of drug in the sample (drug-protein binding and free drug fraction), however, this technique only looks at the free drug fraction in the target tissue. This is a critical CQA for the transdermal patch.

Establishing a different drug product profile using discriminatory skin penetration can help control changes throughout the life cycle.

In order to compare products, it may be necessary to conduct bioequivalence studies, but in some cases, it may be bioequivalence studies that may be discontinued. The composition of the product, on the other hand, can have a profound effect on the functioning and penetration of the skin. The excipient composition of the goods to be compared should be the same or similar in terms of quality and value.

Adequate discriminatory evidence and a report on the development of water penetration testing should be provided in accordance with *in vitro* drug release requirements/disposal procedures.

Human skin from the torso (chest, abdomen, or back) or appropriate at the site of clinical application is selected. Ineffective skin or skin of other species (such as pig, mouse, or guinea pig) may be used if this does not happen. The membrane that is made/made may be suitable in some cases. The leather model used throughout the development process should be forgiven.

It is important to compare items from the same skin type in a comparative test (normal transdermal patch formation or compression composition).

Skin type, repair, and maintenance should all be carefully monitored to ensure that the skin samples used for testing are not damaged and of acceptable quality.

Prior to the start of the test, the integrity of the skin should be checked and shown to be satisfactory. Transepidermal (TEWL) Water Loss Test, measuring triturated water infiltration, and electrical resistance measurement are just a few of the few integrity tests currently available. The effectiveness of the proposed test of integrity, as well as the conditions of admission, should be carefully considered.

A sufficient and appropriate number of repetitions should be included in the test to address skin diversity, conditioning, and skin integrity. In important research, six or more similes are commonly used.

Distribution cells with a skin distribution area of 0.5-2 cm^2 are commonly used in *in vitro* permeation research. For flexible pockets that can be cut to size, such as pond-type strips, spread cells with large areas of skin distribution should be used. Distribution cells should be chemically resistant, sturdy, and easy to handle. It is also important that the distribution cell allows for rapid sampling of the reception and exchange phase while maintaining the integrity of the membrane. Franz (static) distribution cells are the most common, consisting of two cells separated by a skin, either horizontal or vertical. Flow in the cells can also be used, and they are especially helpful in keeping the skin healthy.

The receptor solution used should be as close to vivo as possible. Aqueous buffer is a well-received medium for water-soluble drugs. In the case of water-soluble drugs, solubilizing agents such as surfactants or hydro-alcoholic media such as ethanol/water medium, or proteins such as bovine serum albumin may be used where appropriate. The fluid in the receiver area should come in contact with the skin, which means that there should be no air bubbles under it. Skin integrity should not be compromised by the receptor solution.

Permeation of solubilizing agents from the receptor solution in the skin sample should be considered and avoided. The structure of the receptor medium and the melting studies should be explained to show that immersion conditions can be maintained throughout the study. The active ingredient should be stable in the receiver solution during subsequent testing and analysis.

In every trial, the receiver solution must provide conditions for solubility immersion and ensure that the entry of the active substance is not blocked by the receiver. The maximum

concentration of the active substance in the receiver solution reached during the test should not exceed 10-30 percent of its maximum melting in the receiver solution to be considered an acceptable immersion state. Immersion conditions in stagnant cells can be maintained during testing by changing the recipient category regularly or by using the flow in the device.

Adequate mechanisms must be in place to ensure that the receptor medium is fully integrated with all exposed skin in order to penetrate satisfactorily.

Acquisition of an active object in the receiver area is measured using an assay of consecutively obtained samples of the receiver's liquid to determine the spread of the active substance in the membrane. With the active component content, liquid receptor aliquots can be tested using the HPLC or LC-MS certified system and any other certified t-analyzes.

Skin and sample preparation

The type of skin (origin, type, body part) should be specified, the maintenance and transport of the skin should be properly defined and controlled, and the repair and treatment (firmness, separation) of the skin should be defined and forgiven, to name a few facts.

For the test to work, skin integrity must be established prior to testing and shown to be satisfactory.

The modification of the transdermal patch drug product sample should be described, and its effectiveness should be discussed. In most cases, the piece is carefully cut to size and placed on the skin of the provider's room. The effect of closure should be assessed when using overlays.

The transdermal patch should not be damaged or altered in any way during the testing or processing of the sample.

Study design/study conditions

The following research design is recommended for invasive studies using human *ex-vivo* skin. Any deviation from the proposed test protocol should be fully forgiven.

• Diffusion cell-a type of Franz or flow;

• Receptor phase, mimic *in vivo* conditions that provide effective immersion conditions, degassed, e. g., in an ultrasound bath to prevent the formation of air pockets;

• The material used may be a water barrier and contain suitable lubricants and/or proteins;

• The receptor phase should continue to move and stay in contact with the skin. Motivational speed should be excused;

• Temperature-the surface of the skin, in the distribution cell, is kept at a temperature close to the human skin temperature (32 ° C±1 ° C). The temperature of the skin can be properly determined before the dose is applied using an infrared thermometer;

• Humidity-Excessive moisture associated with the laboratory should be avoided, i.e. above 70% RH and less than 30% RH;

• The integrity of a person's skin should be checked at the beginning of the test;

• Eligibility for integrity testing eg, (TEWL), triple water intrusion, electrical resistance or visual inspection (but not acceptable in critical subjects) and acceptance conditions should be fully discussed;

• Number of repetitions-The selection of the number of samples should be excused in terms of the scope of the test and shown as mathematically appropriate;

• Number of skin donors-at least 2 different donors;

• A region of skin formation-breast (breast, abdomen or back) or corresponding to the area where the application is made;

• The amount of time points should be sufficient to show the permeation profile satisfactorily. A minimum of 5 valid sample time intervals for sample points and an advance time point, based on learning requirements;

• The length of the study period should be justified in relation to the internal management of the use. If the duration of the study is more than 24 h, it should also be indicated that the protective function of the skin and integrity is adequately maintained;

• Non-closed cases, in the event of overlays being used, the impact of the closure should be investigated.

Skin irritancy

The OECD Guidelines for Chemical Testing are updated periodically according to science progress. In such reviews, special attention is given to possible improvements in animal welfare. This updated version of the first guide 406, adopted in 1981, is the result of a meeting of OECD experts in Paris in May 1991 [22].

At present, the relationships of structural-quantity and *in vitro* models have not been sufficiently developed to play an important role in assessing the skin potential of objects to continue to be based on *in vivo* models [22].

Magnusson and Kligman grading scale for the evaluation of challenge patch test reactions

0 = no visible change

1 = discrete or patchy erythema

2 = moderate and confluent erythema

3 = intense erythema and swelling

Studies of cutaneous retention

Specific tests of drug overdose on all skin and previously separated skin layers, as well as the use of reconstructed skin models, can be used to test skin retention of drugs and other bioactive chemicals. Several procedures can be used to separate the skin layers. By immersing the skin in warm water (60 °C) for 1 minute, the dermis and epidermis are separated. The epidermis is then separated from the dermis. The stratum corneum can also be separated by immersing the entire skin in a solution of trypsin 24 h at 37 degrees Celsius. Only stratum corneum is obtained after the skin has been digested with an enzymatic solution [20].

In vitro and *in vivo* skin studies have been used to develop experimental techniques. Following skin penetration tests by diffusion cells, these tests can be performed *in vitro*. *In vivo* experiments were performed on animal models (mice or rats). These inspections should be based on agreements approved by the veterinary commission. In these cases, the formulation is applied to the skin of the animal, and the animals are killed after a certain period of time, and the application area is surgically removed to analyze the drug stored in the skin. The skin is cut into small pieces for drug analysis and maturation in solution for drug withdrawal. Ultra-Turrax homogenizer can be used to make homogenized skin tissues. Using precise and validated analytical procedures, drug evaluations were performed. HPLC is the most widely used analysis method [20].

There is currently a trend in animal testing methods instead, and 3D human *in vitro* reconstructed human models are one of them. Three-dimensional (3D) *in vitro* skin types have been used in educational and industrial research fields to evaluate the toxicity and effectiveness of pharmaceuticals, therapeutic products, and cosmetics, as well as to investigate interactions between the skin and its microbiota. Replacement skin supplements made from keratinocytes and human fibroblasts are commercially available and are recommended to check skin irritation and rust. In addition to their importance in drug testing and drug products, 3D *in vitro* skin models have several limitations as they can mimic the most complex area of all real human skin thickness. Most 3D *in vitro* skin models are composed of basic adult fibroblasts and keratinocytes, with the exception of melanocytes and Langerhans cells, which have different nutritional and physiological needs. *In vitro* skin transplantation has not yet made a significant contribution to drug skin testing. On the

other hand, tissue engineering has shown promise in the development of complex 3D *in vitro* skin models to replace cut-out skin in animals, such as psoriasis models and *in vitro* infection models such as herpes, papillomaviruses, and Candida albicans [20].

***In vivo* evaluation of the skin permeation of drugs**

In vivo skin absorption tests were performed by conducting pharmacokinetic studies or alternatively using cutaneous microdialysis. In both cases, the tests were performed by human volunteers or animal models and by procedures previously approved by the ethics committee [20].

Preclinical pharmacokinetic evaluations

The purpose of this study was to determine whether the target plasma concentration of the drug is achieved or maintained long after transdermal formation. In most cases, the *vivo-vitro* relationship is determined by *in vivo* skin permeation data [20]. In general, pharmacokinetic testing uses plasma concentrations of the drug. For this, blood samples are collected at different times after administration of the transdermal delivery system. Plasma is rapidly separated by centrifugation, and the drug is extracted and analyzed using selected analytical methods, such as HPLC or gas chromatography. In most cases, the use of chromatographic techniques combined with mass spectrometry is required. Pharmacokinetic parameters are determined in the plasma concentration system of drugs compared to time. Recommended limits should be calculated below the curve, the maximum plasma concentration of the drug, and the time required to reach the limit. Drug overdose in a stable condition and duration of intoxication can be determined. Mouse models are commonly used in these studies. Transdermal formation is controlled in the back or abdomen areas of animals. Beagle dogs and guinea pigs are selected from other subjects. Although these pre-clinical trials are important, the results cannot be transmitted to humans if selected animal models show significant differences in skin penetration, drug metabolism, and elimination [20].

Cutaneous microdialysis

The probe is applied to defined skin layers (epidermis or dermis). The health solution (saline or Ringer's solution) is applied slowly using a pump (1–10 µl/min). The compounds in the interstitial tissue fluid are dissolved in dialysate on the probe. The samples were devoid of protein and other macromolecules due to the molecular weight loss of the semipermeable membranes (20-100 kDa). Dialysate is collected at different times, and the drug is analyzed directly by HPLC. However, in some cases, due to the small sample size (few microliters) and drug purification, high sensitivity analysis techniques, such as HPLC associated with mass spectrometry, are required. Drug recovery in the dialysate is an important parameter to be determined.

Drug-related recovery is evaluated using a retrodialysis procedure. In this experiment, a drug solution with a known concentration is used for a limited time. Next, the concentration of the drug on dialysate is determined. Cinitial and Final Focus are used to determine relative acquisition according to the equation below [20].

$$Recovery\ (\%) = 100 - \frac{100 \times C_{final}}{C_{initial}}$$

Hydrophilic compounds can be obtained attractively using Cutaneous microdialysis, however, limitations have been reported in experimental lipophilic drugs. Also, if dialysate is an aqueous solution, the concentration of soluble drugs and lipophilic drugs is much lower. Combined molecular weight compounds, ie proteins and peptides, cannot be determined by cutaneous microdialysis due to cellular weight loss. Overall, this method is attractive because of its low cost compared to the cost of traditional pharmacokinetic research [20].

The following are some of the experimental conditions used for *in vitro* skin permeation tests of the drugs shown in table 5 [20]

Table 5: Experimental conditions used for *in vitro* skin permeation tests of the drugs

Drug (Log Pa)	Receptor medium/ Temperature/Stirring	Skin model	Diffusion cell type	Studied time
Testosterone (Log P = 3.47)	Saline+40% Polyethylene glycol 400/37 °C/NI[c]	Rat skin	Keshary-Chien	26 h
Oxybutynin (Log P = 5.19)	0.9% Saline/37 °C/NI[c]	Skin of Rabbit ear	Franz	8 h
Valsartan (Log P = 4.75)	PBS[b] pH 7.4/37 °C/600 rpm	Skin of Yucatan micropig (dermatomal, Rat and hairless mice and rat.	Vertical	8 h for drug suspensions, and 48 h for transdermal patches
Ondansetron (Log P = 2.07)	PBS[b] pH 7.4/37 °C/NI[c]	Hairless mice skin	Franz	NI[c]
Nicotine (Log P = 0.72)	Saline 0.9%/37 °C/NI[c]	Human skin (200 µm, dermatomated)	Diffusion cell modified with silver-silver chloride electrode	NI[b]
Theophylline (Log P =–0.17) and hydrocortisone (Log P = 1.43)	PBS[b] pH 7.4+0.03% of sodium azide (preservative)/32 °C/Ni[c]	Porcine ear skin	Franz	48 h (theophylline) and 52 h (hydrocortisone)
Diclofenac sodium (Log P = 4.06)	PBS[b] pH 7.4+2.5% hydroxypropyl-β-cyclodextrin/NI[b]/400 rpm	Human skin (only epidermis)	Franz	NI[b]
Donepezil (Log P = 4.71)	PBS[b] pH 7.4/32 °C/600 rpm	Hairless mice and human skin	Keshary-Chien	12 h
Timolol (Log P = 0.68)	Phosphate buffer pH 7.4/37 °C/600 rpm	Guinea pig skin and human skin	Keshary-Chien modified with silver–silver chloride electrode	7h
Donepezil (Log P = 4.71)	Buffer pH 6.0/37 °C/NI[c]	Hairless mice skin	Flow-through cell	24 h
Theophylline (Log P =–0.17) and hydrocortisone (Log P = 1.43)	PBS[b] pH 7.4+0.03% of sodium azide (preservative)/32 °C/Ni[c]	Porcine ear skin	Franz	48 h (theophylline) and 52 h (hydrocortisone)
Fluoxetine (Log P = 4.09)	PBSb pH 7.4+6% of Brij98[d]/37 °C/600 rpm	Hairless mice, rat and human skin	Keshary-Chien	12 h
Nicotine (Log P = 0.72)	PBS[b] pH 7.4/32 °C/600 rpm	Porcine ear skin	Vertical	48 h
Isosorbide dinitrate (Log P =–0.90)	Saline+Polyethylene glycol 400/37 °C/200 rpm	Hairless rat skin	Franz	24 h

[a]ACD/log P values from www. chemspider. com (Royal Society of Chemistry); [b]PBS, phosphate-buffered saline; [c]NI, not informed; [d]Brij98, polyoxyethylene (20) oleyl ether.

CONCLUSION

Pharmaceutical Quality by Design (QbD) is a systematic development approach that begins with pre-defined objectives and emphasizes product and process understanding and process control, based on sound science and risk quality control. Quality by Design (QbD) appears to enhance the guarantee of safe, effective drug delivery to the consumer, and offers the promise of significantly improving production quality performance. The QbD approach reduces overwork during product development, which saves time line and investment. That the QbD method in the transdermal system is most effective in controlling the product development timeline, due to the long process of batch production and the timeline analysis to be completed by developing clusters. Skin/transdermal absorption is a multidisciplinary process, influenced by a number of factors including animal origin and skin type, physicochemical properties of the tested compounds and delivery systems, as well as possible skin treatments and natural features. *In vitro* permeation testing with animal models, with all your limitations, provides essential tools for testing drug delivery systems, skin extensions and drug delivery carriers. Also, these tools make it possible to measure the percutaneous absorption rate of a series of molecules. Although the key features of the *in vitro* skin permeation methodology have been explained in the OECD guidelines, it is clear that the details of the method adopted in some cases need to be adapted. In many cases the ultimate goal of *in vitro* permeation studies is to predict the actual use or behavior.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

There is no conflict of interest from all authors

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