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Improvement of the Experimental Setup for Skin Absorption Screening Studies with Reconstructed Skin EPISKIN®

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Key Words
Human skin model · EPISKIN® · Skin absorption, screening · Permeation

Abstract
Percutaneous penetration studies are usually performed in human skin samples set up in a Franz® cell device. The ability to perform these studies may depend on the availability of skin samples. Reconstructed skin models are an interesting alternative to overcome such limitations but are less easily mounted in diffusion cell devices. Previous data showed that EPISKIN® was a highly performing model to carry out such studies. However, the setup in a PermeGear® cell device is time consuming and therefore unsuitable for screening purposes. Another approach could be using EPISKIN in its cell culture insert. The aim of this study was to compare cutaneous penetration of chemicals applied to EPISKIN samples in a PermeGear cell versus in their own insert. Eight chemicals having widely different chemical structures and penetration potentials were studied. Six test chemicals showed a similar penetration level in both devices. Using the PermeGear cell device, the penetration level was overestimated for the other 2 tested chemicals. The results demonstrated that percutaneous studies with EPISKIN samples could be easily performed using the insert setup. The EPISKIN model has been greatly improved in the recent years and it is now possible to develop screening tests for the evaluation of skin penetration with a higher reliability.

Introduction

Percutaneous absorption studies using human skin ex vivo are a widely accepted alternative to in vivo methods for testing the cutaneous bioavailability of chemical ingredients [1]. The use of such test methods is limited, however, by the number of skin explants available as well as by tricky sample preparation. Usually, skin samples are set up in a Franz® cell device [2, 3]. Furthermore, the interpretation of the data obtained may be difficult due to the variability between biological samples, requiring an increased number of samples to be used [4]. In vitro reconstructed skin models could be an alternative approach to percutaneous absorption studies [5–7]. The production of such models is standardized [6] and therefore improved reproducibility is available compared to ex vivo skin samples, resulting in a reduced number of samples needed for assessment trial. In addition, reconstructed skin samples can be more readily obtained. Thus, they are most useful for screening purposes such as to classify new chemicals according to their cutaneous bioavailability potential. The procedure must, however, be optimized in order to use reconstructed skin for the screening of percutaneous absorption in the same way as the Caco-2 model is applied to evaluate the oral bioavailability potential of a new chemical [8, 9].

Due to fragility, small tissue sample size and other morphological constraints, human reconstructed skin models cannot easily be mounted into static or dynamic

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diffusion cell devices [10]. These devices are commonly used to carry out bioavailability studies with human skin explants. Percutaneous absorption studies with human reconstructed skin models have therefore been performed directly in the cell culture insert [5, 6, 11, 12]. In order to limit any permeant bypass occurring at the interface between the culture insert side wall and the epidermal tissue, the experiments were performed on a skin surface area delimited through gluing a ring onto the epidermis. This setup may, however, lead to inaccurate penetration rates due to an application area smaller than the area in contact with the receptor fluid. Furthermore, imperfect seal of the glued ring could lead to lateral diffusion and possible permeant bypass at the insert-epidermis interface. In-Line cells (PermeGear® Inc., Philadelphia, Pa., USA) [13] were considered as an alternative. In-Line cells allow automated sampling of receptor fluid to be done according to a dynamic protocol. The use of such cells was chosen to lower the product penetration level compared to protocols involving a glued ring, especially for longer exposure times. Thus, mannitol was found to penetrate about 3 times less into the receptor fluid using the dynamic setup compared to the semidynamic setup [13]. Otherwise, using a shorter exposure time (below 6 h), the semidynamic setup did not lead to a higher penetration level. As far as the exposure time was not extended, the penetration level of mannitol would not have been overestimated using EPISKIN® in its original insert. In-Line cells with a dynamic setup could be very useful for kinetic penetration studies over a long exposure time. Despite these advantages, using In-Line cells is time consuming and unsuitable for screening purposes.

Moreover, advances in the EPISKIN production process led us to consider that EPISKIN-insert interface diffusion may no longer exist. The goal of this study was to demonstrate that EPISKIN in its original insert could perform as efficiently as EPISKIN mounted in a PermeGear cell device used in a static protocol with an exposure time set at 4 h. As the finality is to provide experimental conditions of use for EPISKIN in a screening test, such a short exposure time provides several benefits. Firstly, EPISKIN-insert interface diffusion should not occur, allowing an appropriate estimation of the penetration level to be made. Secondly, it prevents problems of chemical stability which may occur with longer exposure times. Thirdly, as shown by previous results obtained on EPISKIN with 7-ethoxycoumarin, 7-ethoxyresorufin and testosterone [14], metabolism increased during the whole exposure time, whereas the penetration rate reached a plateau. Thus, at a longer exposure time (16 h), the proportion of the applied metabolized compound should be increased.

Eight chemicals having widely different chemical structures and penetration potentials were investigated using the same vehicle and they were evaluated in triplicate on each EPISKIN batch using both devices (PermeGear cell vs. insert).

Materials and Methods

Chemicals

2-Nitro-para-phenylenediamine (Nitro-2-PPD; No. 5307-14-2), hydroxyanthraquinone aminopropyl-methyl-morpholinium methosulfate (anthraquinone; No. 38866-20-5), product A, product B, product C and 3,3′-terephthalylidene-10,10′-dicamphosulfonic acid (Mexoryl SX; No. 90457-82-2) were obtained from L'Oréal (Aulnay-sous-Bois, France). Caffeine (No. 58-08-2) was supplied by Fluka (Buchs, Switzerland). Testosterone (No. 58-22-0) was from Acros (Geel, Belgium). 4-Androstene-3,17-dione (4AD; No. 63-05-8) was from Aldrich (Milwaukee, Wisc., USA). All chemicals had a purity level higher than 97%, except anthraquinone (87.5%), and Mexoryl SX was provided as a 33% aqueous solution. Dulbecco-modified phosphate-buffered saline solution (PBS Dulbecco), without calcium, magnesium and sodium bicarbonate, was obtained from Biochrom KG (Berlin, Germany). Tween 80 (No. 9005-65-6) was from Fluka.

All reagents used for chromatography were of analytical grade. Methanol (MeOH) (No. 67-56-1) and acetonitrile (ACN) (No. 75-05-8) (Prolabo, Fontenay-sous-Bois, France) were of chromatography grade. Ultra-high-quality water was obtained from a Milli-Q® system (Millipore, Bedford, Mass., USA). Formic acid (HCOOH) (No. 64-18-6) was from Prolabo, trifluoroacetic acid (TFA) [76–05–1] from Fluka and Pic A reagent from Waters (Milford, Mass., USA).

The same defined simplex formulation was chosen to allow all test products to be soluble at 0.5 or 1 mM. This formulation was buffered either at pH 7 or 9.5 depending on the studied chemical. Table 1 shows the chemical structure, physicochemical properties and experimental conditions used (i.e. donor solution concentration and pH, receptor fluid composition).

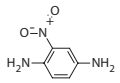
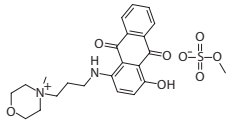
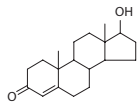
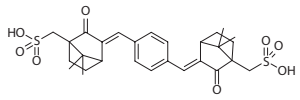
EPISKIN

The reconstructed skin model EPISKIN was provided by EPISKIN SNC (Lyon, France). Upon receipt of the EPISKIN kit (on day 13 of culture), the culture inserts (fig. 1) were removed from their nutrient gel and transferred under aseptic conditions into a sterile culture COSTAR® plate of 12 wells (Corning Inc., Corning, N.Y., USA) containing 1.5 ml of a maintenance medium (EPISKIN SNC) [15] per well. The samples were then incubated at 37°C with 5% CO_2 and at 98% relative humidity. After 24 h, the maintenance medium was replaced by an assay medium (EPISKIN SNC) and stood over night.

Mounting of EPISKIN in the PermeGear Cell Apparatus

PermeGear In-Line cells (PermeGear Inc.) were used under static conditions as previously described [13] (fig. 2). The donor

Table 1. Name, chemical structure, physicochemical properties and experimental conditions (i.e. donor solution concentration and pH, receptor fluid composition) of the test products

	Structure	MW g/mol	log P	Aqueous solubility mg/ml	pKa	pH	Concentration mM	Receptor fluid composition
Nitro-2-PPD		153	0.53[a]	0.20[a]	4.8[a] (B/BH$^+$)	9.5	1	PBS + Tween 80 0.25% (w/w)
Anthraquinone		492.5	0.85[b]	>100[a]	4.8[d] (B/BH$^+$) 8.1[d] (A$^-$/AH)	9.5	1	PBS + Tween 80 0.25% (w/w)
Product A	–	360	2.3[a]	2.21[a]	4.2[a] (A$^-$/AH)	7	0.5	PBS
Product B	–	181	1.47[c]	>0.0166[a]	–	7	0.5	PBS
Product C	–	147	–0.58[b]	52[a]	3.2[d] (A$^-$/AH)	7	0.5	PBS
Testosterone		288	3.32[a]	0.0234[a]	–	7	0.5	PBS
Caffeine		194	–0.07[a]	21.6[a]	–	7	0.5	PBS
Mexoryl SX		562.7	–1.04[a]	330[a]	0.8[d] (A$^-$/AH) 1.4[d] (A^{2-}/A$^-$)	7	1	PBS

B/BH$^+$: base. A$^-$/AH: acid.
[a] Experimental data.
[b] Calculated with Epiwin software.
[c] Calculated with log P SYBIL v. 4.2 software.
[d] Calculated with ACD/pKa DB v. 8.00 software (ACD/Labs).

compartment diameter was 6 mm, providing an application area of 0.28 cm^2. The In-Line cells were placed in a thermostated cell warmer (PermeGear). A Teflon tubing connected the inner and outer compartments of each In-Line cell, providing a static diffusion cell system. Before punching EPISKIN with the 12-mm EPISKIN punch (EPISKIN SNC), a silicon membrane ring, 250 μm thick with an outer diameter of 20 mm and an inner diameter of 14 mm, was placed and centered into the receptor compartment. The ring helps prevent possible squeezing and even damaging of the punched EPISKIN sample after mounting into the diffusion cell. The receptor compartment was prefilled with receptor fluid (cf. table 1 for composition) and the epidermis was centered in the silicon ring. The excess receptor fluid was then removed using cotton swabs and the donor compartment was attached by a clamping system with preadjusted screws.

Use of EPISKIN in Its Original Insert in a COSTAR Plate

The assay medium was replaced with 1.5 ml of receptor solution (cf. table 1 for composition).

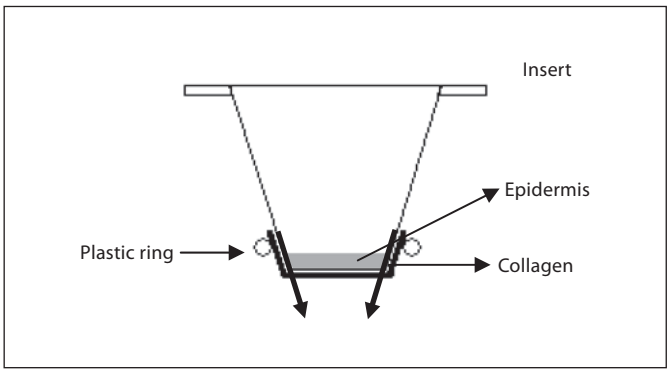


Fig. 1. EPISKIN insert. Possible permeant bypass at the insert-epidermis interface might occur.

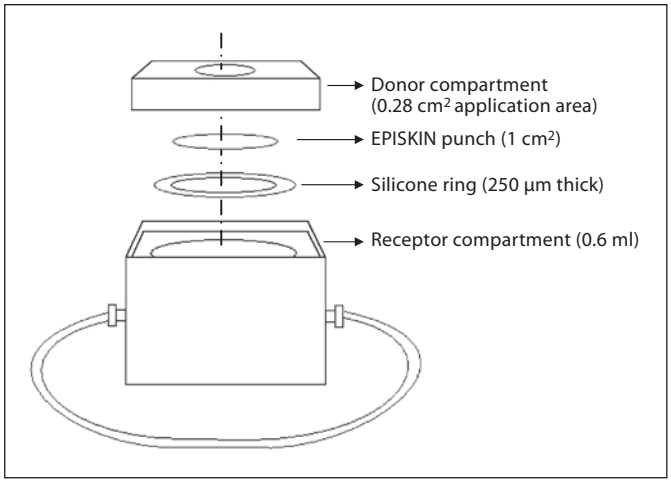


Fig. 2. EPISKIN punch (1 cm^2) mounted into 0.28 cm^2 PermeGear cell using a silicone membrane ring.

Solute Application

The skin samples set up either in the PermeGear cell in the thermostated cell warmer or in the COSTAR plate in an incubator were equilibrated for 1 h. The cell warmer and the incubator temperatures were adjusted to maintain the cutaneous temperature at 32°C. 70 µl of this formulation (i.e. 250 µl · cm^{-2}) was applied to each sample in a PermeGear cell and 250 µl of the same solution to the samples in their insert. Each test product was studied in both devices in triplicate for each batch of EPISKIN. The COSTAR plate was covered during the experiment and was submitted to an agitation of 80 rpm. The reduced exposure time and low donor solution concentration (below 0.05% weight/weight) guaranteed sink conditions. Moreover, the measured aqueous solubility of all studied chemicals (cf. table 1) is enough so that their extent of diffusion was not limited by the composition of the receptor fluid.

Sample Collection

After 4 h exposure time, the receptor fluids were collected and analyzed. For experiments with PermeGear cells, the skin surface was washed with extreme care, twice with 100 µl of 5% sodium lauryl ether sulfate and twice with 100 µl of water; each washing was followed by skin surface drying with 2 cotton swabs. After the In-Line cell dismantling, the receptor fluids were collected and analyzed.

For experiments on samples in their insert, the inserts were carefully removed to avoid any contamination of the receptor fluid by residual donor solution and the receptor fluids were collected and analyzed. No sample preparation was required before analysis except for product C. The samples were diluted with 1 volume of 0.1% formic acid aqueous solution to prevent chromatographic profile distortion.

Analysis

Nitro-2-PPD was analyzed using a spectrophotometer Spectra Max Plus (Molecular Devices, Sunnyvale, Calif., USA) adapted for 96-well plates. Three-hundred microliters of each solution (standards and unknown samples) were distributed in UV transparent 96-well plates. Full spectra were recorded from 260 to 850 nm. The maximum wavelength was set at 550 nm. Two chromatographic systems were used: the Alliance 2695 system (Waters) coupled with a photodiode array detector PDA 996 [Waters, liquid chromatography (LC)/UV; table 2a] and the HP1100 system (Agilent Technologies, Waldbronn, Germany) coupled with an electrospray ionization mass spectrometry (MS) system (ZQ, Micromass, Manchester, UK, LC/MS; table 2b). Isocratic methods were used, since each product was quantified separately. The percentages of the organic and aqueous phases were adjusted in order to obtain a capacity factor between 2 and 5 for each test product. The electrospray ionization mode was used to detect all products analyzed by LC/MS. The electrospray probe was operated at 3.2 kV in the positive ion mode and 2.75 kV in the negative ion mode. The source block and desolvation temperatures were 110 and 250°C respectively. The desolvation gas and cone gas flow rates were 450 and 50 l · h^{-1} respectively. The cone voltage, single-ion recording transition and dwell time were adjusted for each test product. Table 2 describes the analytical conditions for each studied chemical using both systems. The specificity of each analytical method was controlled with a blank sample.

Data Acquisition and Analysis

Nitro-2-PPD data acquisition was performed with SoftMax Pro software (Molecular Devices) and quantitation with Excel (Microsoft, Redmond, Wash., USA). LC/UV data acquisition and analyses were carried out with Millenium v.3.2 (Waters). LC/MS data acquisition and analyses were performed using Mass Lynx v.4.0 and QuanLynx v.4.00.00. Unknown sample concentrations were calculated from the equation $y = ax + b$, as determined by the linear square regression of the calibration line constructed from the peak area of the test product and from the response at 550 nm for Nitro-2-PPD. Mexoryl SX displayed 2 peaks (i.e. *trans-trans* and *cis-trans* isomers); the response factor between isomers 1 and 2 was measured from both calibration lines. Quantitation was done by summing the area of peak 1 and peak 2 and keeping the response factor between peak 1 and peak 2. Sequence analysis was validated by calculating standard accuracy (i.e. measured concentration/nominal concentration · 100), which was between 85 and 115%. Any MS deviation response was controlled by reinjecting a standard solution considered as control quality. The control quality accuracy was between 85 and 115%. Analytical performances (dynamic range and regression coefficient) are listed in table 2a and b for all compounds studied.

The penetrated levels were calculated by dividing the amount of test product found in the fluid receptor by the amount of applied product.

Preparation of Standard Solutions and Simplex Formulation

Standard stock solutions were prepared at 0.5 mM or 100 µg/ml in acetonitrile or receptor fluid. Subsequent dilutions were made in receptor fluid. Stock solutions were kept at 4°C. Except for Mexoryl SX, the purity and counterion were not taken into account, concentrations of standard stock solutions and donor solutions were expressed as test product content. Simplex formulations were prepared and kept at 4°C. Each simplex formulation was quantified; the experimental value was taken into account to calculate the penetrated level.

Table 2. Chromatographic conditions and analytical performance for each of the test products

a LC/UV system

Column	Column temperature, °C	Mobile phase	Flow rate ml/min	Analysis time, min	Injected volume, µl	λ_{max}, nm	Dynamic range, µg/ml	r^2
Anthraquinone (Xterra RP18, 5 µm, 4.6 · 50 mm with precolumn Xterra RP18, 5 µm, 4.6 · 20 mm)	ambient	H_2O + 0.1% TFA/ACN 63/37	1	5	50	554	0.615–49.2	0.9982
Mexoryl SX (Xterra RP18, 5 µm, 4.6 · 50 mm with precolumn Xterra RP18, 5 µm, 4.6 · 20 mm)	ambient	H_2O + Pic A/MeOH 45/55	1	8	50	343	0.035–5.63	0.9998

b LC/MS system

Column	Column temperature, °C	Mobile phase	Flow rate ml/min	Analysis time, min	Injected volume µl	Ionization mode	Cone voltage V	SIR transition	Dwell time s	Dynamic range, µg/ml	r^2
Product A (Xterra RP18, 5 µm, 2.1 · 50 mm)	20	H_2O + 0.1% HCOOH/MeOH 50/50	0.2	6	10	ESI positive	20	361.1	1	0.040–2.0	0.9994
Product B (Atlantis DC18, 5 µm, 2.1 · 50 mm)	45	H_2O + 0.1% HCOOH/MeOH 60/40	0.2	3.5	25	ESI positive	20	182.1	0.5	0.010–0.95	0.9994
Product C (Atlantis DC18, 5 µm, 2.1 · 50 mm)	45	H_2O + 0.1% HCOOH/MeOH 90/10	0.2	4	25	ESI negative	25	146	1	0.032–0.81	0.9994
Testosterone (Atlantis DC18, 5 µm, 2.1 · 50 mm)	45	H_2O + 0.1% HCOOH/MeOH 30/70	0.2	4	25	ESI positive	33	289.2	0.5	0.040–2.1	0.9977
Caffeine (Xterra RP18, 5 µm, 2.1 · 50 mm)	20	H_2O + 0.1% HCOOH/MeOH 85/15	0.2	6	10	ESI positive	25	195.2	1	0.019–1.9	0.9997

SIR = Single-ion recording; ESI = electrospray ionization.

Results and Discussion

The use of EPISKIN in its insert in a COSTAR plate widely simplifies the experimental setup compared to the use of PermeGear cells. The area of exposure (0.28 cm^2 for PermeGear cell and 1 cm^2 for EPISKIN insert) is one of the major differences between the 2 systems. It does not normally modify product diffusion through the skin sample [2, 13]. However, the area increase can lead to a more efficient evaporation of the vehicle which could affect product diffusion. Unlike the PermeGear cell, the COSTAR plate can be covered during the 4-hour exposure time, thus limiting vehicle evaporation. The anthraquinone derivative was then studied with and without cover (table 3). Without cover, the anthraquinone penetrated dose increased more than 3 times. This effect could be due to vehicle evaporation leading both to changes in its composition and to an increase in anthraquinone concentration. However, other results showed that the concentration (between 2.5 and 0.5 mM) did not affect the penetration level (data not shown). Thus, the changes in vehicle composition seems to be the proper interpreta-

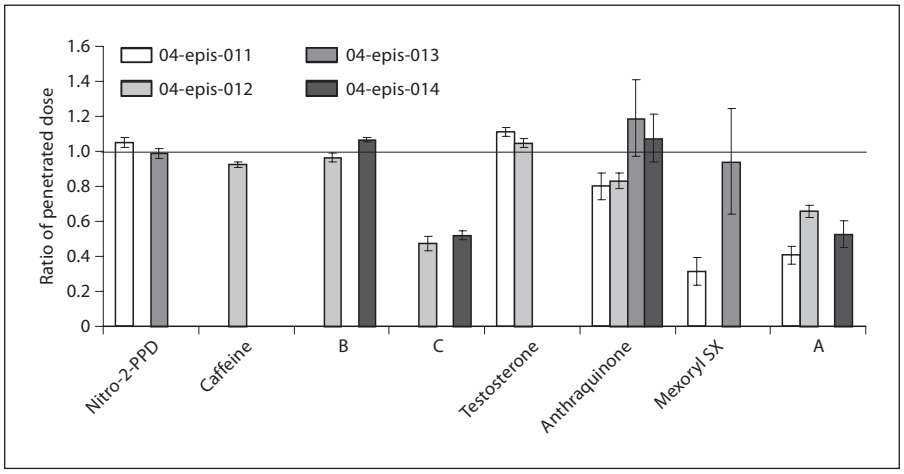


Fig. 3. Ratio of the penetrated dose using insert vs. PermeGear cell for each of the 8 test products and each EPISKIN batch tested.

Table 3. Effect of covered COSTAR plate on penetrated dose of anthraquinone

	With cap	Without cap
Penetrated dose, %	1.60	5.30
Standard deviation	0.24	1.04
Variation coefficient, %	15.0	19.7

tion. Consequently, the use of a cover is recommended to improve the reproducibility of the results by limiting the vehicle evaporation. At least, it guarantees a defined liquid receptor volume.

As previously discussed, when using EPISKIN in its original insert with a low exposure time, EPISKIN-insert interface diffusion should not occur, allowing an appropriate estimation of the penetration level to be made. The 2 dyes (Nitro-2-PPD and anthraquinone) were included in the set of test products to allow EPISKIN-insert interface diffusion to be checked on visually. Either with PermeGear cell or with EPISKIN in its original insert, only the exposed area was colored, demonstrating that no EPISKIN-insert interface diffusion occurred (fig. 1). Experimental data confirmed these observations, as the difference in product penetration level between the 2 methods was negligible (fig. 3).

EPISKIN and other similar reconstructed skin models display metabolic activities. Several types of metabolic activity were investigated: NAD(P)H:quinone reductase [16], glutathione-S-transferase, cytochrome P450 1A1 [17], esterase [18], steroid [19] and aromatic amine [20] metabolism. To evaluate the extent of metabolism in the specified conditions of use testosterone was studied. Reconstructed skin obtained from male cells [15] leads to different metabolic pathways of testosterone compared to cells of female origin [21]. Using EPISKIN, testosterone is mainly metabolized into 4AD. Testosterone was applied to an EPISKIN sample in PermeGear cell configuration as well as in insert device. The proportion of 4AD regarding the penetrated testosterone amount remained small in both cases; 2.8 and 4.0% respectively for PermeGear cell and insert device. Such low a metabolism extent results from the experimental conditions of application and is not modified by the setup.

Figure 3 shows the ratios of the penetrated dose of the test product in the receptor fluid compartment using the insert to the penetrated dose using the PermeGear cell device for the 8 test products. Table 4 displays the inter- and intrabatch mean percentages and their variation coefficients of the applied product found in the receptor fluid compartment. According to table 4, the inter- and intrabatch variability was almost identical with both methods. For the 3 products with the highest penetration rates (Nitro-2-PPD, caffeine and product B), the intra- and interbatch variability was lower than 15%. For Mexoryl SX, which has by far the lowest penetration rate according to in vitro study in human skin [22], the intrabatch variability was significantly higher. Such results explain the variations observed in figure 3 between different EPISKIN batches for Mexoryl SX. Nevertheless, the use of EPISKIN in the original insert did not lead to a higher penetration level.

Products A and C exhibited a particular behavior. Their penetration level was significantly lowered by a fac-

Table 4. Mean penetrated amount of the 8 test products using PermeGear cells or samples in their insert

		PermeGear cell				Insert			
		Intrabatch		Interbatch		Intrabatch		Interbatch	
		mean	CV, %	mean	CV, %	mean	CV, %	mean	CV, %
Nitro-2-PPD	04-epis-011	63	6.3	57	14	66	4.4	59	18
	04-epis-013	52	1.8			51	5.4		
Caffeine	04-epis-012	66	1.7	–	–	61	3.2	–	–
Product B	04-epis-012	50	2.6	57	19	48	4.4	58	26
	04-epis-014	65	2.4			69	2.2		
Product C	04-epis-011	58	10	48	31	27	15	23	25
	04-epis-013	37	21			19	9.1		
Product A	04-epis-011	21	5.2	20	14	8.6	21	11	32
	04-epis-012	22	16			15	9.9		
	04-epis-014	17	12			8.9	26		
Testosterone	04-epis-011	5.1	33	6.1	23	5.7	3.8	6.6	19
	04-epis-012	7.1	4.2			7.5	4.2		
Anthraquinone	04-epis-011	5.2	4.3	5.3	22	4.2	16	5.0	15
	04-epis-012	6.7	7.1			5.6	9.4		
	04-epis-013	4.0	15			4.7	32		
	04-epis-014	5.3	9.1			5.7	22		
Mexoryl SX	04-epis-011	0.98	55	0.73	49	0.31	42	0.38	26
	04-epis-013	0.48	50			0.45	53		

Results are provided for each individual batch and the mean of them. In both cases variation coefficients (CV) are reported. Each product was evaluated in triplicate in each EPISKIN batch and condition.

tor of 2 when EPISKIN was used in its original insert. Applying the PermeGear cell device may have stressed the EPISKIN model during the mounting. Such constraints do not occur using EPISKIN in its original insert. The histological evaluation of treated skin samples shows great differences between the 2 setups. With the PermeGear cell device, the unexposed skin area (i.e. below the donor compartment) is stressed and injured. In contrast, when EPISKIN is used in its original insert, the reconstructed epidermis exhibits a homogeneous appearance. Moreover, the use of the PermeGear cell device requires a handling of the EPISKIN sample by punching it, which increases the stress. The results from products A and C seem to reflect the stress experienced by the reconstructed epidermis. Particular unknown physical-chemical properties of these test products might explain such behavior.

It has been demonstrated that EPISKIN in its original insert allows the throughput compatible with percutaneous screening studies to be reached. For such purposes, the analytical methods used need to be appropriate. LC/MS [23] and particularly the LC/MS/MS system is recommended [24] considering its sensitivity, specificity and versatility of use. The different ionization sources available [25] cover a wide range of chemicals. Similar applications have been developed for absorption, distribution, metabolism and elimination screening in the pharmaceutical industry such as metabolic stability [26, 27], oral bioavailability on CaCo-2 [28] or drug-drug interactions [29]. The LC/MS system used in this study demonstrated its analytical capabilities to be used for percutaneous screening studies with reconstructed epidermis.

Conclusion

Topical application of various chemicals to reconstructed skin has already been performed for other applications (cutaneous irritation [15], testing of skin-tar-

geted androgen modulators [19]). For such purposes, diffusion cells were not necessary. Using the EPISKIN model in its insert for cutaneous penetration studies has allowed the test to be performed at the same level of throughput as other applications while providing the same level of reliability as when mounted in the diffusion cell device. Similar results were already observed by a recent study [7]. The challenge was reached thanks to the improvements made to the EPISKIN model in recent years.

Our results prove that using the EPISKIN model in its insert leads to an accurate measurement of the penetration rate when the exposure time to the test product remains lower than 4 h. On the opposite, the use of PermeGear cells could lead to an overestimation of the penetration rate for some products despite this short time of exposure. For instance, these findings cannot be applied to longer exposure times, although preliminary results indicate that exposure could be increased up to 16 h with EPISKIN in its insert without overestimating the penetration rate.

It now seems possible to develop a rapid screening test method to evaluate cutaneous penetration with a higher reliability. At the very least, the use of reconstructed skin in its insert could permit cutaneous penetration studies to be automated as it has been achieved with the Caco-2 screening system [9]. The results from this study are the first step towards a development of a protocol for screening studies on skin penetration. Such a protocol will have to be validated according to ex vivo human skin data. It may identify new chemicals with high or low absorption potentials in large series and help select the optimal candidate for development.

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