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Evaluation of finasteride skin permeation

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1. PURPOSE OF THE TEST

To compare the in vitro permeation through the human epidermis and the retention into the membrane of finasteride contained in:

- gel containing liposomes (lipos gel);
- gel based on Carbopol (carb gel);
- gel based silicone + liposom (sil lip gel).

The experiments were performed by using modified Franz diffusion cells.

2. EQUIPMENT

Modified Franz cell system (self-made apparatus). These cells had a wider vertical column than the original Franz-type diffusion cell and the bowl shape was removed. They had a diffusion area of 0.636 cm^2 and 3.0 ml (approx.) receiver capacity. The receiver volume of each cell was individually calibrated.

GC analysis.

3. METHODS

Skin preparation

This permeation study was performed by using human epidermis.

One single patient was used so as to eliminate intrasubject variability and was prepared following a standard protocol. Samples obtained from the abdominal skin of human donor (30-50 years old, Eurasiatic) who underwent cosmetic surgery was used as a membrane between the two chambers in the Franz Cells. Within 6/8 h after removal the excess fat was carefully removed from the full-thickness skin. The skin sections were cut into squares of about 2.5 x 2.5 cm, sealed in aluminium foil and frozen at -20°C. Prior to preparation, the skin was thawed to room temperature and, after immersing the skin in water at 60°C for 1 min, the epidermis was gently separated from the remaining tissue with forceps and left to dry.

Evaluation of skin integrity

Before being mounted on the Franz Cell, the epidermis was carefully visually inspected to avoid any possible defects. Moreover, the conductivity of the isolated epidermis was measured to ensure the integrity of the barrier membrane. In particular, epidermis samples with a resistivity above 15 $k\Omega cm^2$ were used for experiments.

Skin permeation study through human epidermis

The upper and lower parts of the Franz cell was sealed with Parafilm® and fastens together by means of a clamp, with the membrane acting as a seal between the donor and receptor compartments.

The skin was carefully mounted on the lower half of the Franz cell with the dermis facing downwards and the stratum corneum side in contact with the formulation. At the beginning of the experiment, semisolid formulation with the help of an excavated silicon cylinder was applied on the skin as donor phase (approximately $100mg/0.636cm^2$). The receiver compartments were filled with a saline solution filtered through a filter membrane with pore size 0.2 μm. Before use, this solution was sonicated under vacuum to remove air; special care was taken for the absence of air bubbles between the receptor medium and the dermis in the receptor compartment. The prepared Franz cells were continuously stirred with a small magnetic bar and were kept at 37°C with a circulating water bath, throughout the experiment, so that the membrane surface temperature was 32 ± 1°C. Only the receptor compartment was in contact with the circulating water at 37°C. At predetermined times over 24 hours (1/3/5/7/24 h), 0.2 ml samples were withdrawn from the receiver compartment and replaced with fresh receiver medium. Sink conditions were maintained throughout the experiments. Three replicates per test preparation was performed. The withdrawn samples were assayed directly by GC to determine the concentrations of the compounds that have permeated through the epidermis.

Finasteride retention into human epidermis

Modified Franz diffusion cells were disassembled; the human epidermis samples were carefully removed from the cells after 24 h following application of the formulations. Residual gel on the surface of the membrane was removed and the membrane was washed with 10 ml methanol to remove the excess of substance that was not permeated through the epidermis. The membranes were cut into small pieces and collected in different tubes containing 5 ml methanol. The samples were sonicated for 2 h and left to stand for 24 h. After 24 h the supernatant was filtered through a filter membrane with pore size 0.45 μm and analyzed by HPLC with the selected method.

Analysis of finasteride by LC/MS-MS

The method for the determination of finasteride was developed by a LC/MS-MS technique. The instrument used is a mass spectrometer Varian 320 model equipped with Ion source ESI + Pump 212-LC (Varian), Software MS Workstation 6.9.1. version.

Analyses were carried out in Multiple Reaction Monitoring mode injecting directly the samples derived from the cells of Franz and building a calibration curve for quantitative determinations.

In the Table the precursor (373.2) and product ions are reported:

Finasteride MRM:

Parent Q1	Q3	Capillary	Collision energy	Type
373.2	305.3	85.0	21.5 V	Qualitative ion
373.2	317.3	85.0	14.0 V	Quantitative ion
373.2	373.2	85.0	9.5 V	Qualitative ion

For quantitative determination the transition 373.2 $\rightarrow$ 317.3 was used.

ESI + parameter:

- Needle Voltage: 5550 V
- Shield Voltage: 600 V
- Nebulizing Gas (N2) Pressure: 40,00psi
- Drying Gas (N2) Pressure: 50,00 psi
- Drying Gas (N2) Temperature: 400°C
- Manifold Temperature: 42°C
- API Housing temperature: 63.9°C
- Q0 Offset:-4.0V
- L4 Offset:-1,0V
- Elettromultiplier: 1550,0 V

Chromatographic conditions:

Mobile phase A: formate buffer 3 mM;

Mobile phase B: Acetonitrile;

Flow rate: 200µL/min

Gradient:

Time(min)	% phase A	% phase B
0	90	10
2	90	10
7	10	90
12	10	90
14	90	10
17	90	10

Column: Kinetex 2.6µ C18 100A 100x2.1mm (Phenomenex)

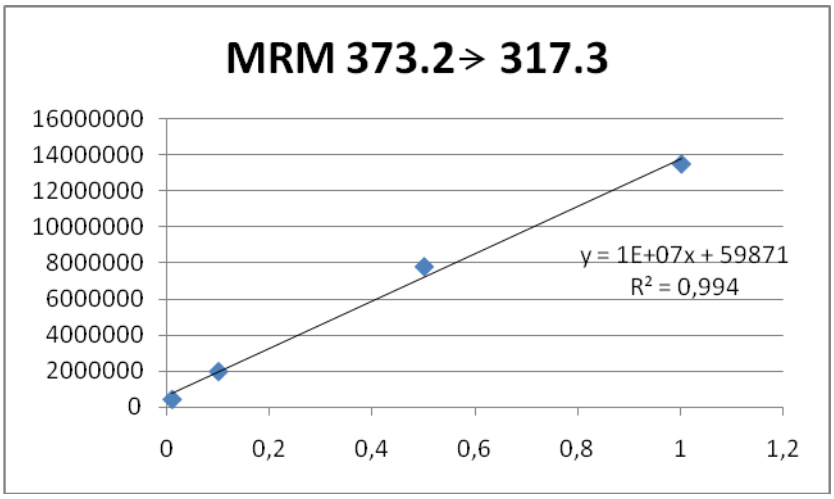
Injection volume: 20µL

Column oven temperature: 40°C

For quantitative analysis, a calibration curve was built as follows, using the transition 373.2 → 317.3:

Standard solutions for setting up the calibration curve:

- Std 1: 1.00µg/mL;
- Std 2: 0.50 1µg/mL;
- Std 3: 0.10µg/mL;
- Std 4: 0.011µg/mL

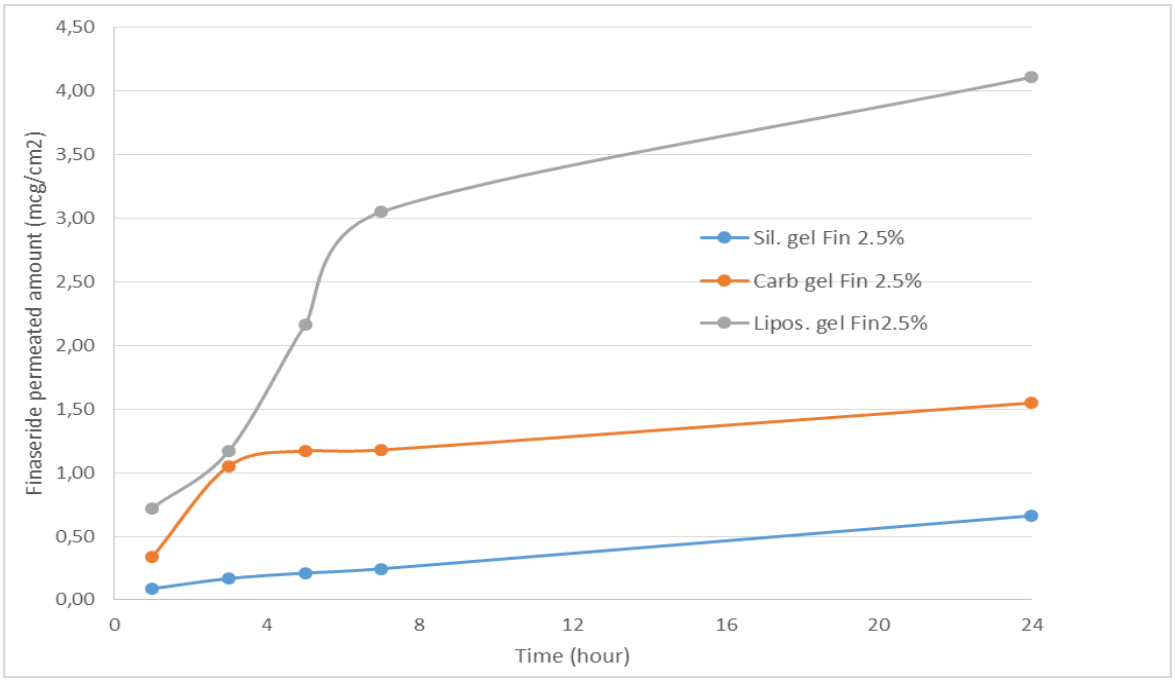


4. RESULTS

Skin permeation study

The finasteride permeation profiles through human epidermis obtained by using as donor phase each gel are reported in Figure 1.

Figure 1 - Finasteride permeation profiles through human epidermis



The finasteride permeated amounts after 24 h and the fluxes (Table 1) were statistically different.

In order to compare in vitro and in vivo data, the equation reported below was used to calculate the steady-state plasma concentration of finasteride estimated on the base of in vitro permeation data:

Css = (A × J) / CL

Css for Finasteride cps 1 mg = 9,2 ng/mL

Where A is the skin area available for diffusion, J is the in vitro permeation rate (flux) of finasteride (µg/cm2*h), and CL is the systemic clearance after oral administration (165 mL/min). The calculated Css value was compared to Cmax at steady state (9.2 ng/mL) obtained after oral administration, 1 mg daily per os.

Assuming as application area of the gel the value of 200 cm^2 and knowing the clearance value, it is possible to estimate the plasma steady-state concentrations (Css) from the in vitro experiments conducted with the human skin from the same donor.

Table 1 – Permeation parameters measured through human epidermis by using the gels.

	Permeated amount after 24 h (µg/cm^2)	Permeated amount after 24 h □□)	Flux (µg/cm^2/h)	Css (ng/mL)	Retained amount after 24 h (µg/cm^2)	Retained amount after 24 h □□)
Sil lip gel	0.66 ± 0.24	0.016	0.02 ± 0.00	0.49	0.41 ± 0.28	0.081
Carb gel	1.55 ± 0.06	0.034	0.04 ± 0.01	0.74	0.34 ± 0.21	0.066
Lipos gel	4.11 ± 1.50	0.241	0.13 ± 0.01	2.69	0.49 ± 0.39	0.095

Css for Finasteride gel 2,5% Sil-lip gel = 0,49 ng/mL
Css for Finasteride gel 2,5% Carb gel = 0,74 ng/mL
Css for Finasteride gel 2,5% Lipos gel = 2,69 ng/mL

AREA 200 cm2

The retained amounts of finasteride into human epidermis after 24 h, reported in Table 1, are not statistically different.

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