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- TRATTAMENTO CELLULITE
- Medical Device class IIa
- Notified Body: ISTITUTO SUPERIORE DI SANITA’

LABORATORI FARMACEUTICI KRYMI ROMA

1. EFFICACY TEST

1A. DETERMINATION OF THE QUANTITY OF WATER ABSORBED BY A HYPERTONIC EMULSION (Attachment 1)

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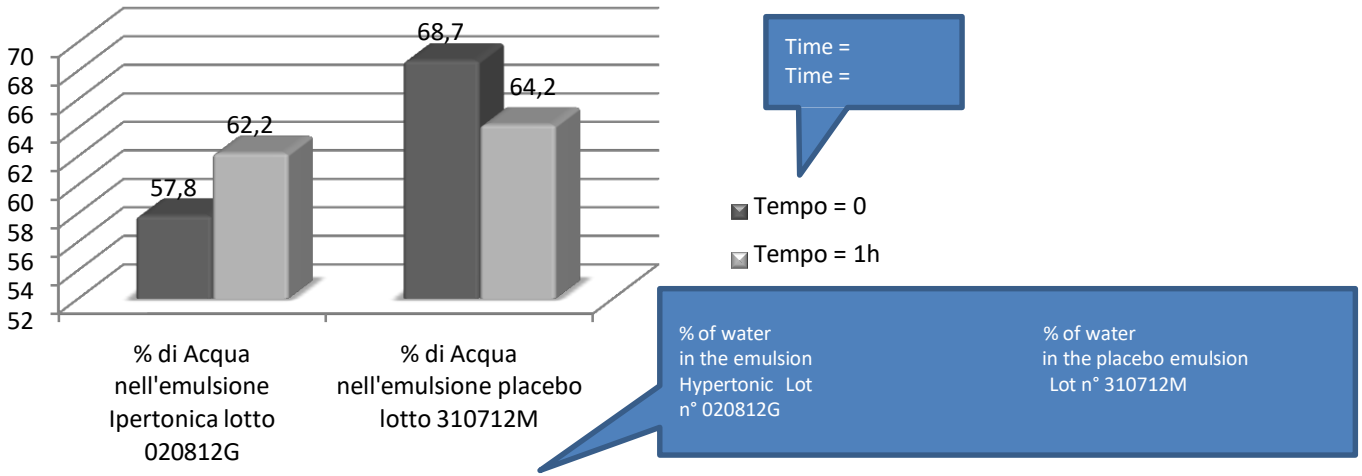
The Department of Pharmaceutical Sciences of the University of Milan was requested to conduct a study to evaluate the actual capacity of the emulsion to encourage elimination of water through the skin. The purpose of the study was to evaluate "*ex-vivo*" the quantity of water absorbed by the hypertonic emulsion vs. a placebo emulsion.

RESULTS

The results prove that, unlike the placebo emulsion, the hypertonic emulsion successfully moves the water placed under the membrane outwards towards the skin surface. One hour after application on the membrane in the adopted conditions, the water content of the hypertonic emulsion increases by 5%, while that of the placebo emulsion drops by about 4%.



1. EFFICACY TEST

1B. SKIN COMPATIBILITY CHECK OF TWO COSMETIC PRODUCTS WITH ANTICELLULITE ACTIVITY AFTER APPLICATION IN NORMAL CONDITIONS OF USE: EVALUATION OF EFFICACY AND COSMETIC PROPERTIES

Double blind application test with dermatological control

(Attachment 2 and 2.1)

A study coordinated by Prof. Leonardo Celleno was conducted at the company Evic Italia to:

-verify skin compatibility of two cosmetic products with anticellulite action, after application for 4 consecutive weeks in normal conditions of use;

- evaluate and compare anticellulite efficacy and cosmetic properties.

The study was conducted in double blind on 30 volunteers for 28 days. It was then decided to extend the study to 56 days on a group of 10 volunteers.

RESULTS

The results are reported in the attached study.

1. EFFICACY TEST

2B. SKIN COMPATIBILITY CHECK OF TWO COSMETIC PRODUCTS WITH ANTICELLULITE ACTIVITY AFTER APPLICATION IN NORMAL CONDITIONS OF USE: EVALUATION OF EFFICACY AND COSMETIC PROPERTIES

XII. CONCLUSIONS

With the experimental conditions adopted in this study, the product called Cream A - Code 201112M presented good skin compatibility after application in normal conditions of use.
Moreover, the product presented good anti-cellulite activity.
Specifically, the results obtained with instrumental measurements highlighted the fact that treatment with the investigational product induced a reduction in panniculus adiposus thickness and thigh circumference, and an increase in skin elasticity.
Said results were confirmed by the clinical trial conducted by the investigator, which revealed an improvement in the orange peel appearance of the skin and in the figure in about half the subjects.
Even the opinions of volunteers confirmed the good anti-cellulite activity of the investigational product, which was particularly appreciated because it:
- induces a visible reduction in the orange peel effect
- has good moisturising efficacy
- makes the skin silkier
- makes the skin smooth and compact.

The product was also appreciated for its cosmetic qualities.

2. TOXICITY STUDIES

2a. CITOTOXICITY-DIRECT CONTACT ON (Attach 3)

SUMMARY

On the test product "CREMA ANTICELLULITE" was carried out a toxicological study aimed to evaluate any cytotoxic effects.
The following test was performed:

- cytotoxicity direct contact according to ISO 10993-5:2009

For the **cytotoxicity test by direct contact**, a confluent BalbC 3T3 cell culture in exponential phase of growth was used.
A qualitative evaluation was performed observing cell culture by an inverted microscope, while a quantitative evaluation was performed using the Neutral Red Uptake method (NRU).
The NRU is a method that allow to measure cell vitality using their capacity to incorporate and to bind a cellular vitality dye, the Neutral Red.

The test product was applied to the monolayer of BalbC 3T3 and was incubated at 37°C ±1°C in CO_2 atmosphere for 24 hours.
After 24 hours of incubation the cells were observed to microscope (qualitative evaluation) to evaluate the biological reaction.

After 24 hours of contact, in the wells treated with test product a zone limited to area under specimen shown malformed or degenerated cell (reactivity grade 2).

After the qualitative evaluation cells were treated for 3 hours with the Medium containing the cell vitality dye and then with a Desorb Solution that allows to obtain a cell lysate. The optic density was than calculated after a 540nm spectrophotometric reading.

Cells treated with test sample have shown a cell vitality reduction of 23.54%.

On the basis of the results, interpreted according to ISO 10993-5:2009, the test product "CREMA ANTICELLULITE" must be considered **NON CYTOTOXIC.**

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2. TOXICITY STUDIES

2b. SKIN IRRITATION TEST (Attach 4)

On the test item "CREMA IPERTONICA" a toxicological study was carried out to evaluate the possible local toxic effects, throughout the following test:

- skin irritation test according to ISO 10993-10:2010

The **skin irritation test** was carried out through a semi-occlusive application; the test item was applied on the intact skin of 3 rabbits, in the dorsal region both on the left and on the right side.
Each animal had the right caudal region and left cranial region treated with the test item. The right cranial region and the left caudal region were treated with a no irritant humidified gauze (25mm x 25 mm), used as control.

Reactions were evaluated 1 hours following the removal of the patches and were evaluated again at 24, 48 and 72 hours after exposure.

In treated regions no oedema or erythema have been observed.

In control regions no oedema or erythema have been observed.

PRIMARY SKIN IRRITATION INDEX: 0,0

On the basis of the results, interpreted according to ISO 10993-10:2010, the test item "CREMA IPERTONICA" must be considered **NOT IRRITANT** for skin.

2. TOXICITY STUDIES

2c. Assessment of allergic contact dermatitis potential through the reduced Local Lymph Node Assay (rLLNA) (Attach 5)

This LLNA assay is carried out according to the OECD 429 guideline (2001) [1] and to ESAC statement on the Reduced Local Lymph Node Assay of 27th April 2007. The LLNA assay is foreseen by the UNI EN ISO 10993-10 rule as alternative to the classic Magnusson & Kligman test (appendix E.2) [2].

Aim of the test is to evaluate the sensitising potential of a cosmetic product or medical device using an in vivo test, the *reduced Local Lymph Node Assay* (rLLNA) according to the OECD 429 guideline (2001) [1] and to ESAC statement on the Reduced Local Lymph Node Assay of 27th April 2007.

RESULTS:

No signs of general toxicity were observed. No animals had any weight loss. Locally, no signs of irritation were observed in any animal, including the DNFB treated mice. This last observation is likely due to the relatively low concentration (0,02%) of DNFB we used. DNFB showed an evident sensitising effect, as expected. In the following tables the obtained dpm and SI values are shown.

In the above experimental conditions, the product:

did not show any skin sensitizing potential.

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