Final Report

EVALUATION OF THE ELASTICITY, FIRMING AND MOISTURIZING EFFICACY OF A COSMETIC PRODUCT ON 20 VOLUNTEERS (LONG TERM TEST)

STUDY N°	**KH264/14-02**
SPONSOR	**LABORATOIRE DR PAUL ET KARIN HERZOG SA** **Route de Taillepied, 1** **1095 Lutry - SWITZERLAND**
SAMPLE	**Vitamin H** **Batch: 431 1014**
REPORT DATE	**04//08/2014**
REPORT N°	**REL/1423/2014/CLI/SAB**

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AUTHENTICITY OF RESULTS

I hereby declare that the study concerned by this report was carried out under my responsibility, according to the experimental protocol and the quality plan of Abich S.r.l. I also state that, where applicable, all procedures were compliant with the principles of Good Clinical Practice.
All relevant observations and data recorded during the test are reported in this study report.
I certify the re-reading of this report and I do agree with its content.

The Study Director

Dott. Samuele Burastero

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SUMMARY

By assignment from the Company **LABORATOIRE DR PAUL ET KARIN HERZOG SA**, on the test substance **Vitamin H Batch: 431 1014** an *in vivo* test has been carried out in order to evaluate its capability in improving the skin elasticity, firming and hydration on 20 healthy volunteers.

For this purpose, the following parameters were investigated:

1) Elasticity and firmness by means of **Cutometer®: STUDY N° KH264/14-02 A**

2) **Corneometry,** that is the measure of skin hydration based on the determination of the capacitance of the stratum corneum surface (by means of Corneometer ® CM285): **STUDY N° KH264/14-02 B**

The measurements with **Cutometer®** were carried out on the volar surface of the right and left forearm area before (T0) and 30 days after the bi-daily application of the product; the right forearm was treated with the product while the left one remained untreated.
The evaluation of moisturizing efficacy was performed with **Corneometer** ® CM285 were carried out on the volar surface of the right and left forearm before (T0) and 30 days (T30) after the bi-daily application of the product.
Moreover at the end of this period the participants to the study, filled in a questionnaire relative to a subjective evaluation of the cosmetic pleasantness, of the organoleptic characteristics, of the perception of efficacy and to a general satisfaction of the product and its performances .

The study was performed at the Abich Cosmetic Lab. in Via Bruno Buozzi, 4 – 20090 – Vimodrone (Milan), Italy.

The experimentation started the 19/06/2014 and ended the 21/07/2014.

DISCLAIMER

According to COLIPA guidelines, the test was performed with the assumption that the Sponsor under its responsibility provided to the personnel of Abich Clinical and Cosmetological Trials Center truthful information on any ingredient of the test product endowed with potential toxicological relevance. On the basis of such information, a general assessment of the toxicological information concerning the product was preliminarily carried out and ethical implications as to its use during the present study have been considered

REGULATORY ASPECTS

This study has been carried out in compliance with the most recent recommendations of the World Medical Association Declaration of Helsinki- ethical principles for medical research involving human subjects (Helsinki Declaration 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and according to the Colipa Guidelines for the evaluation of the efficacy of cosmetic products (May 2008).

ARCHIVING

The clinical study protocol, the corresponding raw data and the final report are kept in the archives of Abich Testing Centre, in Via Buozzi, 4, 20090-Vimodrone (MI), both in electronic format and in reduced paper format for a period of 10 years from the issue of the final report. The control samples of the test substance and eventual specific reference material will be kept at last for 3 month, or more, if requested by the Sponsor.

TEST SUBSTANCE

The test substance consists of a yellow emulsion.

Name:	**Vitamin H**
Batch/ Formule code:	**431 1014**
Sample Code Abich:	4419/14-01
INCI composition:	see annex
Pao / Expiration date:	n.a.
Storage conditions:	room temperature

The characterization of the test substance is under responsibility of the Sponsor.

PANEL RECRUITMENT

a) Characteristics of the panel

The study was performed on 20 female volunteers, aged from 18 to 65 , who were identified from the database of volunteers of the Abich Test Centre, and who were evaluated as appropriate for participation in the study and not suffering from diseases to the skin areas to treat.
Before the beginning of the study each volunteer has read and signed an informative form (informed consent form, C.I.). Each volunteers has had the opportunity to ask any kind of questions regarding the study to which was given an exhaustive answer. The volunteer was explained the aim of the test, the procedure and the possible risks related.
Only after signature of the informed consent the participation in the study was permitted.
Only volunteers in good general health conditions were included in the study.
The originals of these informed consent forms were archived at the Abich Cosmetic Lab. All subjects signed a consent allowing to treat personal data according to the Italian law (privacy. D.Lgs 196/2003).

Table 1: Volunteers participant to the study.

N°VOLUNTEER	CODE	AGE
1	ROVA262	52
2	STSC335	43
3	GIGA455	52
4	TIRA307	48
5	DOCA447	54
6	ANPAN13	48
7	MACO533	23
8	ARHU534	23
9	DOFE535	23
10	ELMO399	19
11	SEAN395	22
12	LADA394	22
13	LUTE528	60
14	GITO475	22
15	MACA268	55
16	GITA212	24
17	SAPR495	24
18	LAMA184	39
19	GAAP211	31
20	CADA238	41
MEAN		**36,25**

b) Exclusion criteria

The following criteria of exclusion were applied:
- Pregnancy or nursing condition.
- Medication (local and/or systemic) which might interfere with the test evaluation.
- Subjects with signs of irritation at the application site.
- Subjects with dermatological problems which that might interfere with the study.
- Simultaneous participation to other studies, which that might interfere with the test evaluation.

Moreover, after study start, the following withdrawal criteria were applied:
- Volunteers who did not follow the conditions as described in the informed consent form, C.I;
- Volunteers who suffered any illness or accident or developed any condition which could affect the outcome of the study;
- Volunteers who did not longer wish to participate in the study.

c) Criteria for study withdrawal

After study start, the following withdrawal criteria were applied:
-Volunteers who did not longer wish to participate in the study;
-Volunteers who during the study suffered any illness or accident or developed any condition which could affect the outcome of the study;
-Volunteers who did not follow the conditions as described in the Study Protocol.

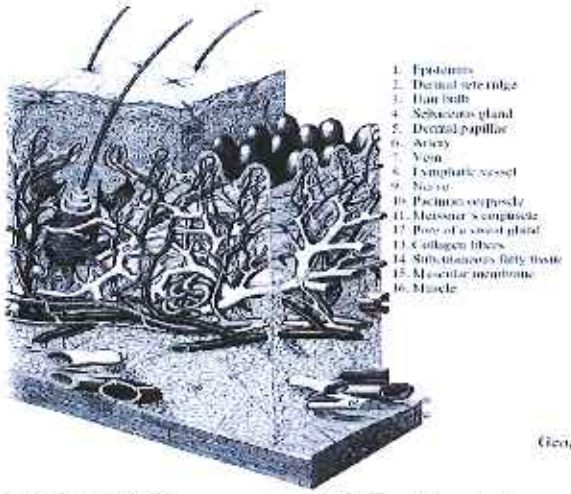
STUDY N° KH264/14-02 A

IN VIVO EVALUATION OF ELASTICIZING AND FIRMING EFFICACY OF A COSMETIC PRODUCT ON 20 VOLUNTEERS
(LONG TERM TEST)

1. INTRODUCTION

The skin is made up of three components: epidermis, dermis, and hypodermis. The epidermis consists of four layers and is completely regenerated in a 28-day cycle known as skin turnover time.
The outermost layer of the epidermis (stratum corneum) consists of the NMF (Natural Moisturizing Factor) and of a sebaceous film, which cover the epidermis and prevents moisture from evaporating. The dermis is the main component of the skin and consists of collagen and elastin, which maintains the firmness and elasticity of skin, respectively, as well as of hyaluronic acid and water. Hyaluronic acid is a jelly-like substance filling gaps between collagen and elastin.
In the aging process changes in the connective tissue take place that are not easily detected until a secondary manifestation appears. Early changes in visco-elastic properties include sagging and increased extensibility. These changes are measurable in vivo, and represent quantitative parameters that can serve as markers for the efficacy of specific cosmetic products with anti-aging properties. There are four major components in the skin that contribute to determine the viscoelastic parameters, namely the collagen fibers, the elastin fiber, the proteoglycans and water. Each of these components has a unique effect by itself, but their interaction generates very complex responses.
Collagen, the protein that can be found in the greatest quantity in the body, forms the structural network in the skin. Its primary constituents are the amino acids glycine, proline and hydroxyproline. Its mechanical strength (it is indeed on of the one of the strongest proteins in nature) accounts for skin resistance and robustness. Collagen is perceived to begin to deteriorate as we grow older, leading to continuous reduction in the skin's thickness and ultimate sagging.
Elastin and collagen are similar proteins up to some extent. However, elastin is less stretchable than collagen and provides the matrix that binds the individual skin cell. Two unique proteins contained in elastin fibers are desmosine and isodesmosine, which cooperate to enable the skin to stretch and return to its original shape. Skin elastin progressively breaks down as one grows older, leading to the development of wrinkles.
Glucosamino-glycans (GAGs) are special sugar molecules containing glucosamine hydrochloride, N-acetyl glucosamine, and glucosamine sulfate that cooperate to entrap large amounts of water. These component form polysaccharides such as hyaluronic acid, keratin sulfate, heparin, heparin sulfate, dermatin sulfate, and chondroitin sulfate. GAGs are composed of repeated disaccharide units made up of sugars and hexosamines attached to the core of the protein. The GAGs are strongly hydrophilic molecules because they contain large amounts of hydroxyl, carboxyl and sulphate groups, and they physiologically form porous, hydrated gels. Hydrated GAGs cushion the skin by providing mechanical support.

Figure 1 – Skin and subcutis with relative skin annexes.



Geo, 1/1988

2. INSTRUMENTATION AND MATERIALS

The following instrumentation and materials were used:

• **Cutometer® MPA580:** digital probe for instrumental measurement of the cutaneous mechanical parameters, produced by Courage-Khazaka GmbH (Germany).

• **Environmental Thermohygrometer** (Courage-Khazaka GmbH - Germany).

• **Demographycal pencil and tape** measure to delimitate the treated areas and identify the areas where measurements were made.

3. EXPERIMENTAL DESIGN

3.1 Structure of the study

The study has been executed with an open observational modality.

3.2 Aim of the study

The present study is designed to evaluate the effect of the product under study on the skin elasticity and firmness after 30 (T30) days of bi-daily application of the product.
For this purpose, the following biomechanical parameters were investigated before the beginning of the study (T0) and after 30 days.

- **Resistance to deformation** (max elongation measured) of the skin after suction (named R0); R0 represents the passive force of the skin, and in proportional to the compactness/firmness of skin. *The lower this value, the higher is the compactness skin.*

- Skin capability to return to its initial position after undergoing tensile stress (suction). This very important parameter is generally called **gross elasticity** (named R2); R2 quantifies skin deformation and give suggestions of the elasticity of the skin.
  The more this value is close to 1 (or 100%), the more conserved is the elasticity of the skin.

The evaluation implied the comparison between the elasticity and firmness of the area of interest prior to the product application (time 0= T0) with the same parameters detected in the same area at each measurement time.

3.3 Environmental conditions

The study has been carried out in standard environmental conditions, for each measurement time point, by monitoring and maintaining constant the environmental temperature and humidity.

3.4 Treated areas

Each volunteer applied the product twice a day (morning and evening) on the volar surface of the right forearm, between wrist and elbow and on the entire face.

3.5 Evaluated skin areas

The measurements of the hydration were carried out in quadruplicate for each subject for each experimental time (T0 and T30) at the level of the treated (right forearm) and untreated areas (left forearm). The four skin points analyzed at different times were as much as possible superimposable for each time of analysis.

4. ESSAY METHODOLOGY

4.1 Study duration

The study had a total duration of 30 days for each volunteer. The product under study was applied twice a day (morning and evening).

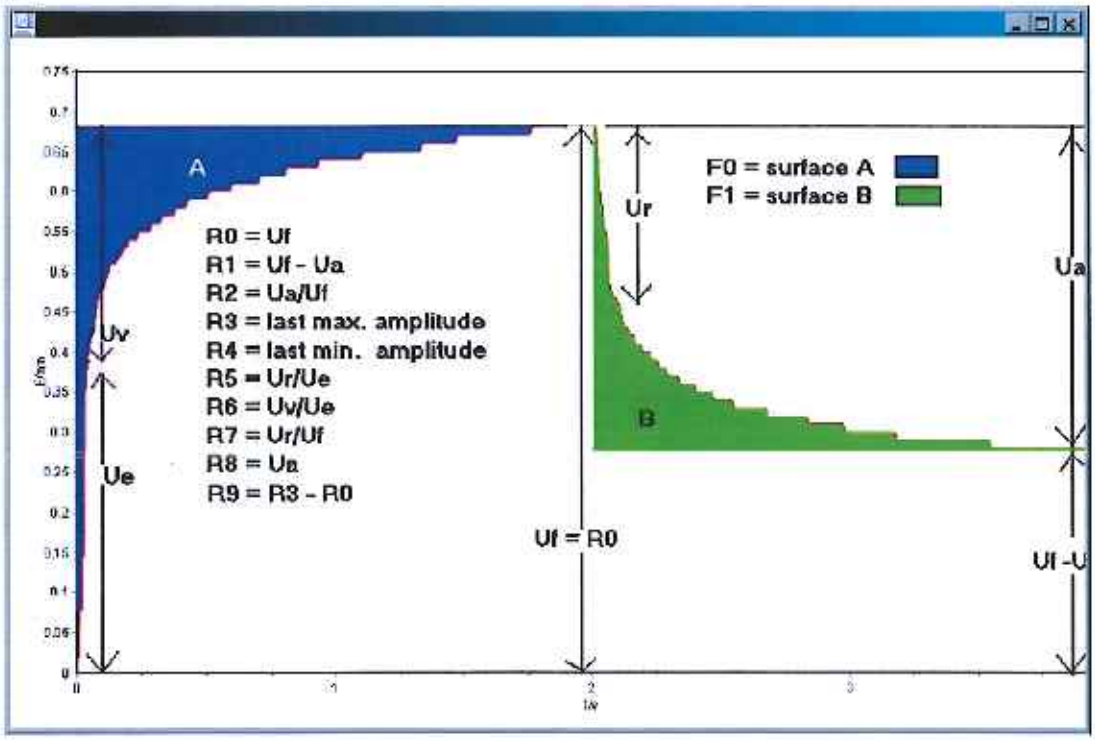
4.2 Preparation of the volunteers

Before each measurement with the Cutometer®, each volunteer was allowed to relax for approximately 10 minutes in an air-conditioned room to avoid anomalous sampling due to excessive sweating or stress.

4.3 Measurement of skin mechanical parameters (elasticity/firmness)

The measuring probe (2 mm in diameter) of the Cutometer® was positioned vertically inside the marked area of skin to be tested.
Then, a negative pressure gradient equal to 450 mbar was created (suction), provoking skin penetration inside the probe opening (range: 1 to 10 mm). Suction time was set to 2 seconds. Release time was also set to 2 seconds (standards as suggested by the Manufacturer).
Each measurement was performed throughout three consecutive cycles (suction + release, Fig.2):
R0 represents the max elongation of the skin after suction;
R2 represents the elastic return of the skin to its initial position after suction.

Figure 2



6. MEASUREMENTS OF ELASTICITY AND FIRMNESS (END-POINTS)

The elasticity and firmness efficacy were evaluated by measuring the skin elasticity and firmness of each subject at time T0, before the beginning of the study and after 30 days (T30) of bi-daily product application. The elasticity and firmness measurements were carried out on the treated and untreated area of each volunteer.

7. STATISTICAL ANALYSIS

All the values relative to the analyzed parameters were gathered for each participant and for each measurement time (see annex).
The % variations of the two parameters were calculated for each volunteer (see annex) and the average % variations were evaluated for each measurement time VS T0 (T30 vs T0 Tables 2 and 4).
The distribution of the values obtained during the measurements at the various experimental times were compared with intra-group analysis using Student's t test (T30 vs T0); P values<0.05 were considered significant.
All the raw data for each volunteers are listed in annex.

8. RESULTS

Under the adopted experimental conditions, the product under examination **Vitamin H Batch: 431 1014** has demonstrated efficacy in improving skin firmness (R0) and skin elasticity (R2) at the level of the analyzed skin area.

In particular, R0 **(firmness)** resulted improved by an average value equal to 8.46% after 30 days of product bi-daily application in comparison with the R0 mean value measured at T0 (basal value), before the beginning of the study. These variations were all statistically significant ($p<0,05$).

The same parameter monitored at the level of the untreated area was increase of a mean value equal to 1,11% after 30 days of treatment respect to the R0 **(firmness)** mean value measured at T0.

R2 **(elasticity)** resulted improved by an average value equal to 3.50% after 30 days of product bi-daily application in comparison with the R0 mean value measured at T0 (basal value), before the beginning of the study. These variations were all statistically significant ($p<0,05$).

The same parameter monitored at the level of the untreated area was decrease of a mean value equal to 0,79% after 30 days of treatment respect to the R2 **(elasticity)** mean value measured at T0.

R0 (firmness)

The tables below report the means of R0 on the panel of 20 volunteers at each observational times (T0, and T30, table1) and the mean % variation values of the same parameter calculated as arithmetical average of the single % variations of each volunteer (table 2).
A decrease of this parameter indicates an increase of skin firmness.
The mean R0 value variations are moreover represented in form of graphs (Graphs 1-2).

Table 1

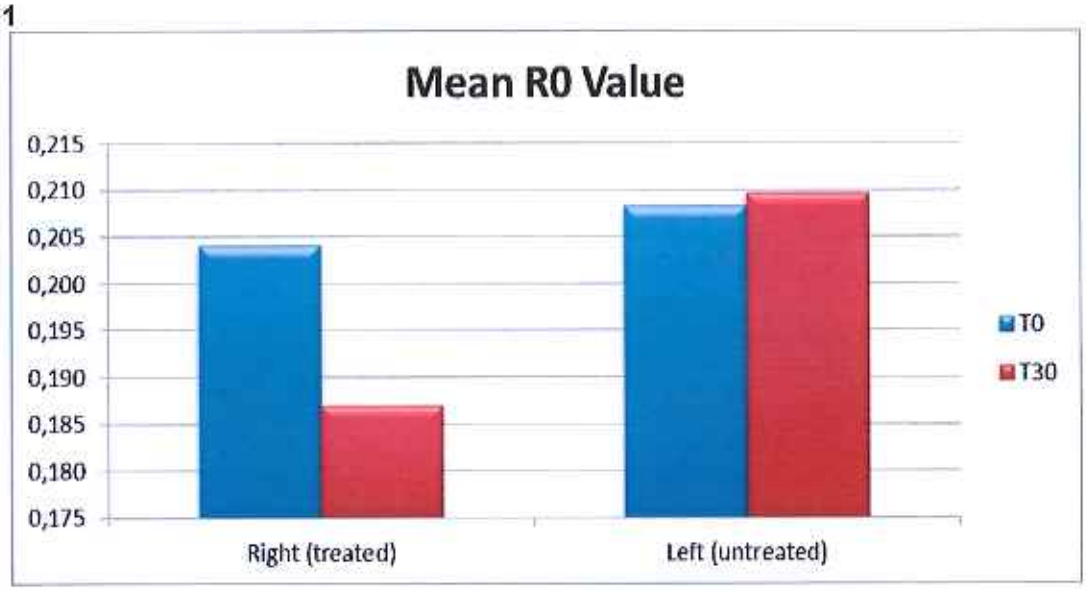
TIME	Right (treated)	Left (untreated)
T0	0,204	0,208
T30	0,187	0,210

Table 2

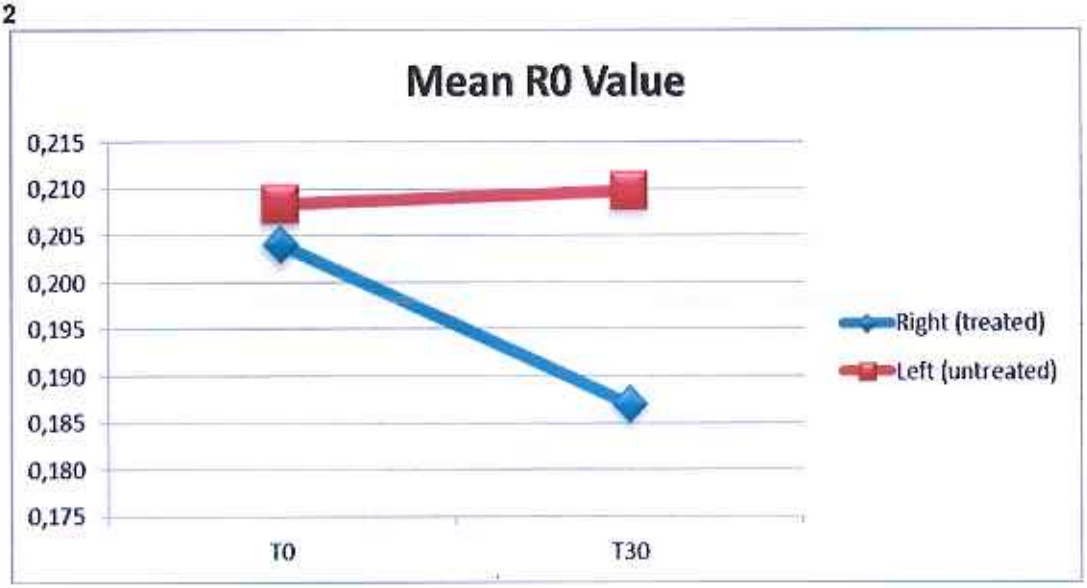
TIME	Mean % variations		p Value	
	Right (treated)	Left (untreated)	Right (treated)	Left (untreated)
T0	-8,46%	1,11%	0,0031*	0,8159

**P values considered statistically significant.*

Graph 1



Graph 2



R2 (elasticity)

The tables below report the means of R2 on the panel of 20volunteers at each observational times (T0, and T30, table 3) and the mean % variation values of the same parameter calculated as arithmetical average of the single % variations of each volunteer (table 4).
An increase of this parameter indicates an increase of skin elasticity.
The mean R2 value variations are moreover represented in form of graphs (Graphs 3-4).

Table 3

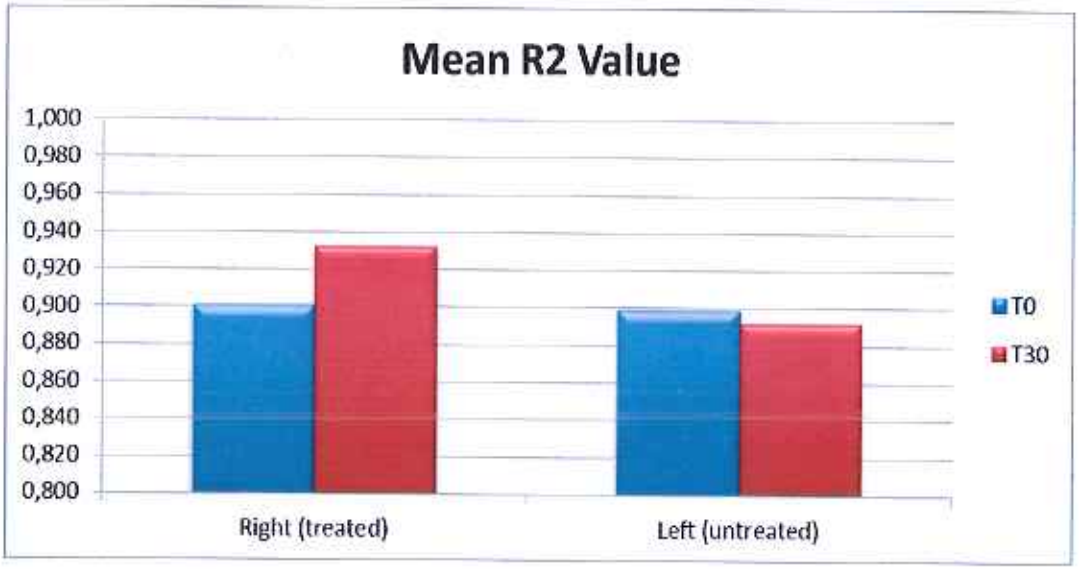
TIME	Right (treated)	Left (untreated)
T0	0,901	0,899
T30	0,932	0,892

Table 4

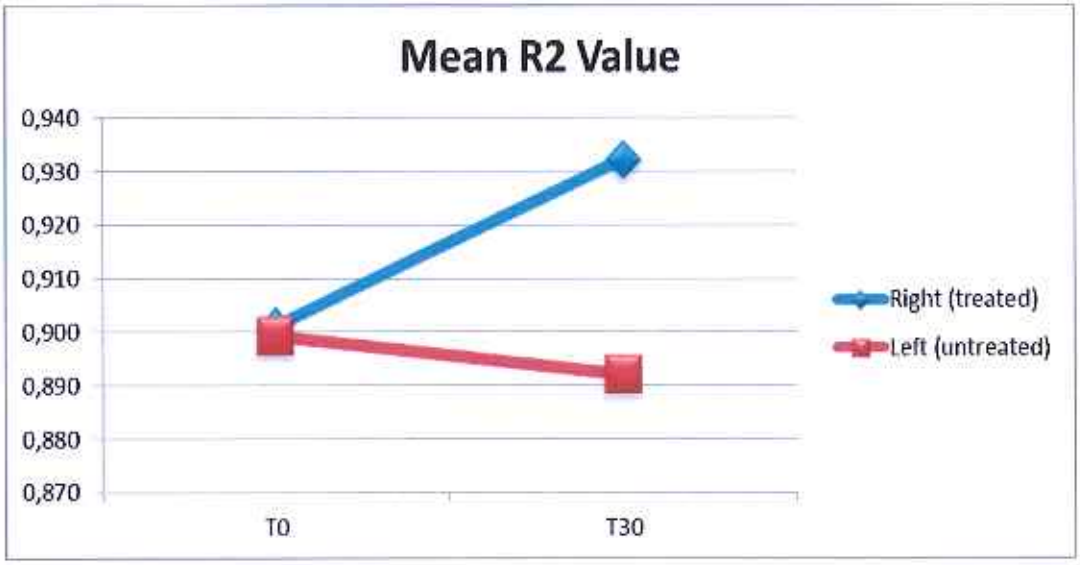
TIME	Mean % variations		p Value	
	Right (treated)	Left (untreated)	Right (treated)	Left (untreated)
T0 VS T30	3,50%	-0,79%	2,56695E-06*	0,0905

**P values considered statistically significant.*

Graph 3



Graph 4



9. DISCUSSION AND CONCLUSIONS

On the basis of the results obtained under the adopted experimental procedure it is possible to conclude that the product under examination

Vitamin H Batch: 431 1014

in the subjects that undergone the test, determined an improvement in skin firmness (R0) and in skin elasticity (R2) of the treated area after 30 days of bi-daily product application;
In particular after 30 days of product bi-daily application **skin firmness** resulted improved by a mean value equal to 8,46% while **skin elasticity** resulted improved by a mean value equal to 3.50%.

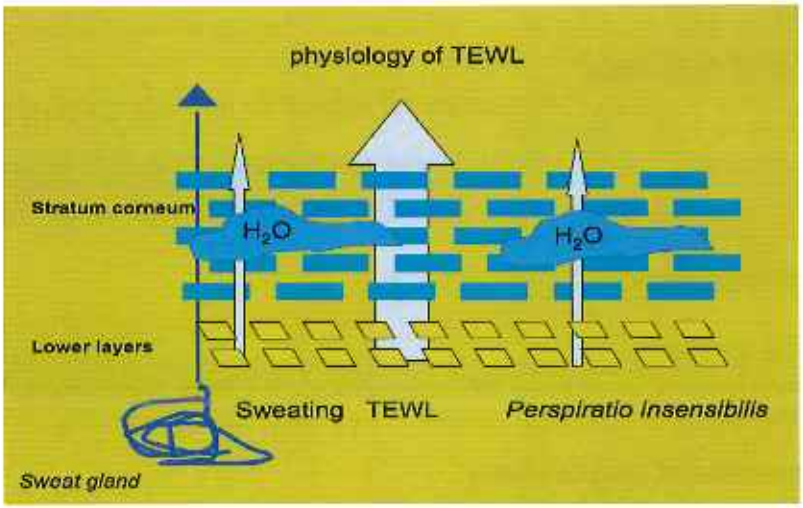
STUDY N° KH264/14-02B

EVALUATION OF THE MOISTURIZING EFFICACY OF A COSMETIC PRODUCT ON 20 VOLUNTEERS (LONG TERM TEST)

1. INTRODUCTION

Water is indispensable for the physiological functions of the skin, in particular of the *stratum corneum* of the epidermis, and the intra-tissue distribution is tightly controlled (Verdier-Sevrain and Bonte, 2007). Water retention by the epidermis depends on two principal factors: 1) natural hygroscopic agents between the corneocytes, collectively indicated as natural hydrating factors; 2) intercellular lipids of the *stratum corneum*, organized in such a way to constitute a barrier to water loss (TEWL, Trans Epidermal Water Loss). The two main components of TEWL are the *perspiratio insensibilis* and sweating (figure 1).
The water content of the *stratum corneum* is correlated to its maturation and desquamation. The increase in TEWL impairs the enzymatic functions required by the physiological desquamation and determines the typical aspect of dry and dehydrated skin.
TEWL is a very sensitive indicator of the skin barrier integrity and it can be measured instrumentally by means of evaporimetry. TEWL refers to the total share of water which is lost from the dermal and epidermal tissues to the outside through the stratum corneum.
Alterations of the integrity of skin barrier function are highlighted by elevated values of TEWL while not altered states or improvements of skin barrier are indicated by a baseline values or a decrease of TEWL.
Glycerol is one of the best characterized endogenous components with hydrating function. Furthermore, hyaluronic acid, traditionally considered a structural constitutive part of the dermis, is actually also extremely important in regulating the equilibrium of water content and the barrier function of the epidermis (Rawlings and Harding, 2004). Crucial morpho-functional elements in the control of the skin hydration are the "tight junctions" or "zonula occludens" (Brandner et al., 2006; Proksch et al., 2008), areas localized between two cells with their membranes which are tightly associated to form an impermeable barrier for fluids in vertebrates (corresponding to the septal junctions of the invertebrates). Finally, in this context, acquaporine 3, a transport protein for water and glycerol with epidermal localization also plays a critical role (Boury-Jamot et al., 2009) by affecting the proliferation and differentiation of keratinocytes.

Figure 1



Corneometry is a scientifically recognized method for the measurement of skin hydration, based on the determination of the capacitance of the superficial *stratum corneum*. Similarly to other bio-physical parameters of the skin, this measurement is depending on a number of physical environmental factors, such as humidity and temperature, which must be strictly controlled. The ideal measurement conditions are 20°C and 50% relative humidity, respectively. The seasons and the climate influence the level of skin hydration and for this reason, during the winter time average skin hydration is lower. Also, the use of detergents may dehydrate the skin and has to be considered among the factors that are potentially interfering with the correct execution of the skin hydration measurements.

2. EXPERIMENTAL DESIGN

2.1 Structure of the study

The study has been executed with an open observational design.

2.2 Objectives

This study was aimed to evaluate the effect of the product under examination on the skin hydration after 30 days of twice-a-day application on the volar surface of the forearm. The evaluation implied the comparison of the levels of hydration of the analyzed area at T30 with the levels of hydration measured at T0, before the beginning of the study.

2.3 Environmental conditions

The study has been carried out in standard environmental conditions, for each measurement time point, by monitoring and maintaining constant the environmental temperature and humidity.

2.4 Treated areas

Each volunteer applied the product twice a day (morning and evening) on the volar surface of the right forearm, between wrist and elbow and on the entire face.

2.5 Evaluated skin area

The measurements of the hydration were carried out in quadruplicate for each subject for each experimental time (T0 and T30) at the level of the treated (right forearm) and untreated areas (left forearm). The four skin points analyzed at different times were as much as possible superimposable for each time of analysis

2.6 Mode of application

The product was applied in non occlusive epicutaneous application massaging until absorbed. The application has been carried out in laboratory.
The amount of product applied in each application was approximately 2 mg/cm^2, according to the Colipa Guidelines .

3. ASSAY METHODOLOGY

3.1 Study duration

The study had a total duration of 30 days for each volunteer. The product under study was applied twice a day (morning and evening).

3.2 Preparation of the volunteers

Before each measurement with the Corneometer® , each volunteer was allow to relax for approximately 10 minutes in an air-conditioned room to avoid anomalous sampling due to excessive sweating or stress.

3.3 Measurement of the average skin hydration

The measurement of the skin hydration by means of the Corneometer® CM285 (Courage-Khazaka GmbH, Germany) is a widely accepted technology recognized by the scientific "evidence based" biomedical research (Couteau et al., 2006; Dal'Belo et al., 2006; Heinrich et al., 2003; Leonardi et al., 2002; Li et al., 2001). The instrument determines the capacitance of the epidermis, which is proportional to its aqueous content, at a depth of approximately 15 µm. This method is based on the difference between the relative electric permittivity (often inappropriately defined as the "relative dielectric constant", a non dimensional number) of water (=81) and that of other substances (<7). The probe of the Corneometer® (surface of 49 mm^2) is positioned vertically on the skin area to be submitted to the test, preliminarily identified with a pencil. The measure of the capacitance lasts one second. Each measurement is executed in four replicates and the mean value is recorded.
The values are expressed by the instrument in arbitrary units ranging from 20 to 120. The accurateness of the measurements is ± 3%.

4. INSTRUMENTATION AND MATERIALS

The following instrumentation and materials were used:

- Multiprobe Adapter Systems MPA® (Courage-Kkazaka), equipped with eight probes managed by a single software, among which the Corneometer® CM285, used for the present study;
- Environmental Thermohygrometer (Courage-Khazaka GmbH - Germany).
- Demographycal pencil and tape measure to delimitate the treated areas and identify the areas where measurements were made.

5. MEASUREMENTS OF HYDRATION (END-POINTS)

The hydrating efficacy was evaluated by measuring the skin hydration of each subject at time T0, before the beginning of the study and after 30 days (T30) of bi-daily product application. The hydration measurements were carried out in quadruplicate on the treated and untreated area of each volunteer, respectively, and the means of the measurements were extrapolated for each experimental time.

6. STATISTICAL ANALYSIS

The distribution of the values obtained during the measurements at the various experimental times for the product treated area and the untreated area were compared with intra group analysis (T30 versus T0) and inter group analysis (Treated VS Untrated at each experimental time) through two-tailed, student test for paired data,
Values of $p<0.05$ were considered significant.

7. RESULTS

In the adopted experimental conditions, the product under examination

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has demonstrated efficacy in enhancing the mean hydration of the skin at the level of the application area. In particular, the hydration level is resulted increased by an average value equal to 28,72% after 30 days of application respect to the hydration mean value measured at T0; this variation was statistically significant ($p<0,05$).

The same parameter monitored at the level of the untreated area was decrease of a mean value equal to 0,29% after 30 days of treatment respect to the hydration mean value measured at T0.

The tables below report the means relative to the hydration values expressed in arbitrary units on the panel of 20 volunteers (Table 1), the percentage variations of the mean hydration value at T30 versus the mean hydration value at T0 and P values of t Student's test for intra-group analysis (Table 2), and inter-group analysis (table 3).
The same data have been graphically plotted in figures 2 and 3 of the following pages.

Table 1
Mean hydration values, expressed in AU

TIME	R (treated)	L (untreated)
T0	35,64	36,58
T30	44,72	36,00

Table 2
Percentage variation of the mean hydration values at each experimental time VS T0 and P values of Student's t test .

TIME	Mean % variations		p Values	
	R (treated)	L (untreated)	R (treated)	L (untreated)
T30 vs T0	28,72%	-0,29%	2,46822E-09*	0,5351

**P values considered statistically significant.*

Figure 2
Mean hydration values, expressed in AU

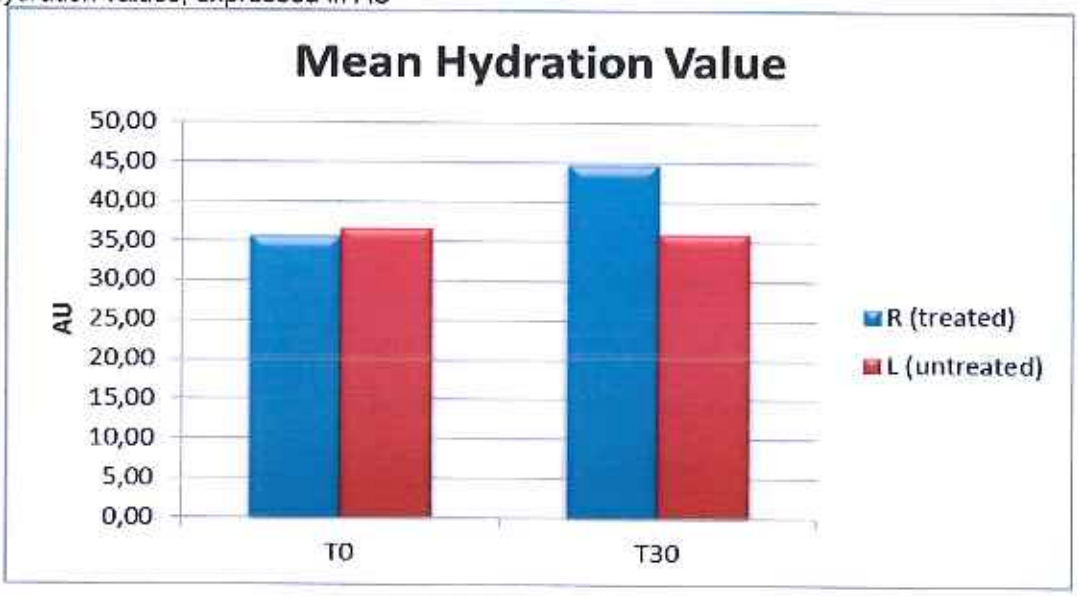
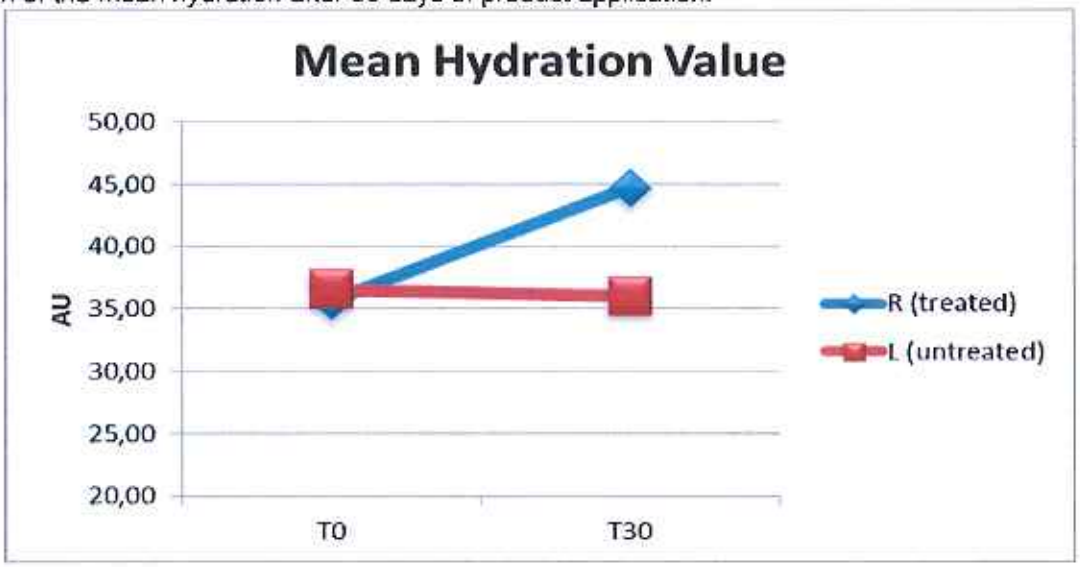


Figure 3
Variation of the mean *hydration* after 30 days of product application.



8. DISCUSSION AND CONCLUSIONS

On the basis of the results obtained with the adopted experimental procedure it is allowed to conclude that the product under examination

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in the test subjects has shown effectiveness in increasing the mean hydration of the treated skin area.

In particular the hydration of the treated area was increased by an average value equal to 28,72% after 30 days of product bi-daily application.

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Declaration WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

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ANNEXES

ANNEX 1

Raw data of R0 parameter (in mm)

N°	COD VOL	T0		T30		% variations T30 vs T0	
		Right (treated)	Left (untreated)	Right (treated)	Left (untreated)	Right (treated)	Left (untreated)
1	ROVA262	0,267	0,257	0,200	0,287	-25,09%	11,67%
2	STSC335	0,249	0,263	0,230	0,266	-7,63%	1,14%
3	GIGA455	0,221	0,240	0,191	0,250	-13,57%	4,17%
4	TIRA307	0,197	0,210	0,171	0,213	-13,20%	1,43%
5	DOCA447	0,212	0,210	0,171	0,213	-19,34%	1,43%
6	ANPAN13	0,177	0,162	0,148	0,150	-16,38%	-7,41%
7	MACO533	0,122	0,147	0,126	0,190	3,28%	29,25%
8	ARHU534	0,224	0,254	0,224	0,253	0,00%	-0,39%
9	DOFE535	0,241	0,254	0,224	0,300	-7,05%	18,11%
10	ELMO399	0,255	0,267	0,266	0,215	4,31%	-19,48%
11	SEAN395	0,231	0,253	0,265	0,236	14,72%	-6,72%
12	LADA394	0,192	0,213	0,168	0,184	-12,50%	-13,62%
13	LUTE528	0,187	0,194	0,210	0,145	12,30%	-25,26%
14	GITO475	0,177	0,187	0,143	0,218	-19,21%	16,58%
15	MACA268	0,215	0,208	0,199	0,204	-7,44%	-1,92%
16	GITA212	0,194	0,188	0,174	0,19	-10,31%	1,06%
17	SAPR495	0,147	0,131	0,122	0,133	-17,01%	1,53%
18	LAMA184	0,163	0,151	0,142	0,157	-12,88%	3,97%
19	GAAP211	0,211	0,206	0,193	0,210	-8,53%	1,94%
20	CADA238	0,199	0,172	0,172	0,180	-13,57%	4,65%
MEAN		0,204	0,208	0,187	0,210	-8,46%	1,11%

Raw data of R2 parameter

N°	COD VOL	T0		T30		% variations T30 vs T0	
		Right (treated)	Left (untreated)	Right (treated)	Left (untreated)	Right (treated)	Left (untreated)
1	ROVA262	0,856	0,853	0,895	0,822	4,56%	-3,63%
2	STSC335	0,908	0,863	0,922	0,857	1,54%	-0,70%
3	GIGA455	0,940	0,901	0,980	0,863	4,26%	-4,22%
4	TIRA307	0,903	0,899	0,979	0,889	8,42%	-1,11%
5	DOCA447	0,892	0,872	0,908	0,844	1,79%	-3,21%
6	ANPAN13	0,819	0,833	0,904	0,840	10,38%	0,84%
7	MACO533	0,926	0,923	0,935	0,886	0,97%	-4,01%
8	ARHU534	0,913	0,897	0,957	0,882	4,82%	-1,67%
9	DOFE535	0,918	0,890	0,953	0,909	3,81%	2,13%
10	ELMO399	0,949	0,943	0,951	0,925	0,21%	-1,91%
11	SEAN395	0,924	0,928	0,958	0,940	3,68%	1,29%
12	LADA394	0,947	0,953	0,976	0,973	3,06%	2,10%
13	LUTE528	0,832	0,840	0,847	0,852	1,80%	1,43%
14	GITO475	0,915	0,924	0,956	0,940	4,48%	1,73%
15	MACA268	0,915	0,907	0,927	0,904	1,31%	-0,33%
16	GITA212	0,875	0,924	0,899	0,912	2,74%	-1,30%
17	SAPR495	0,866	0,886	0,906	0,881	4,62%	-0,56%
18	LAMA184	0,917	0,904	0,926	0,899	0,98%	-0,55%
19	GAAP211	0,882	0,912	0,910	0,906	3,17%	-0,66%
20	CADA238	0,924	0,931	0,955	0,917	3,35%	-1,50%
MEAN		0,901	0,899	0,932	0,892	3,50%	-0,79%

ANNEX 2

Raw data of hydration level (in AU)

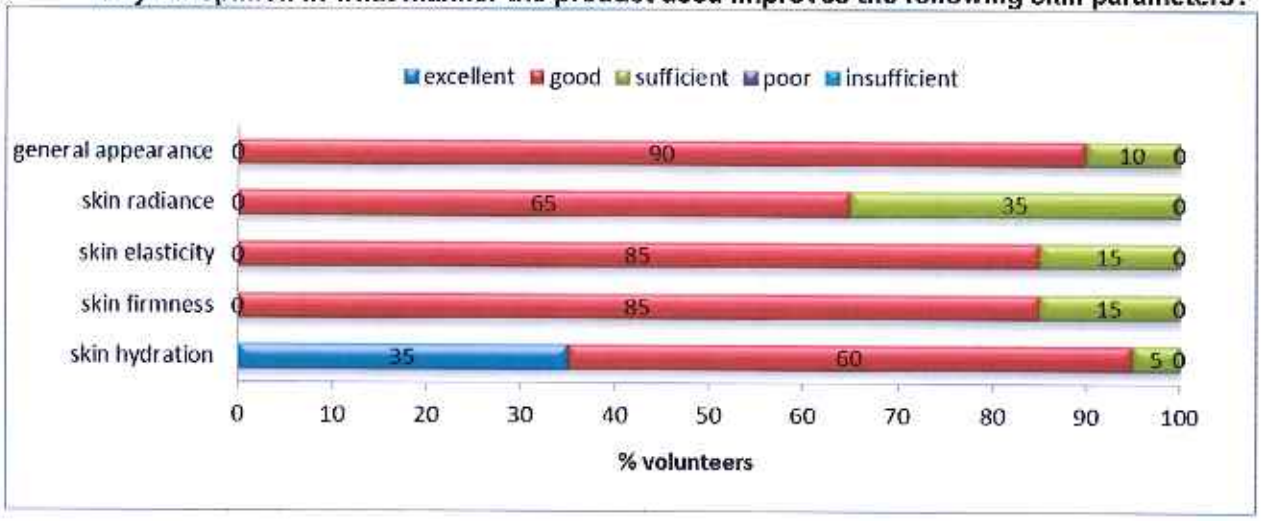
N°	COD VOL	T0		T30		% variation T30 vs T0	
		R (treated)	L (untreated)	R (treated)	L (untreated)	R (treated)	L (untreated)
1	ROVA262	34,85	35,18	48,42	36,92	38,94%	4,95%
2	STSC335	25,60	27,87	34,15	28,44	33,40%	2,05%
3	GIGA455	24,60	25,27	34,38	21,30	39,76%	-15,71%
4	TIRA307	49,57	50,70	51,90	43,82	4,70%	-13,57%
5	DOCA447	34,45	35,70	49,17	40,08	42,73%	12,27%
6	ANPAN13	43,30	46,58	61,17	49,78	41,27%	6,87%
7	MACO533	48,93	46,00	56,98	41,30	16,45%	-10,22%
8	ARHU534	56,03	57,27	60,05	47,60	7,17%	-16,88%
9	DOFE535	44,27	46,63	49,85	39,65	12,60%	-14,97%
10	ELMO399	33,98	37,65	47,98	43,10	41,20%	14,48%
11	SEAN395	41,52	43,30	45,45	43,10	9,47%	-0,46%
12	LADA394	44,97	45,67	51,10	47,10	13,63%	3,13%
13	LUTE528	20,80	23,32	30,30	27,98	45,67%	19,98%
14	GITO475	17,98	20,53	25,95	22,80	44,33%	11,06%
15	MACA268	31,18	30,58	40,18	30,07	28,86%	-1,67%
16	GITA212	22,84	23,47	31,52	22,15	38,00%	-5,62%
17	SAPR495	40,84	39,51	51,94	40,01	27,18%	1,27%
18	LAMA184	19,64	20,08	27,53	20,60	40,17%	2,59%
19	GAAP211	40,28	39,66	51,22	38,24	27,16%	-3,58%
20	CADA238	37,17	36,55	45,21	35,94	21,63%	-1,67%
MEAN		**35,64**	**36,58**	**44,72**	**36,00**	**28,72%**	**-0,29%**

ANNEX 3

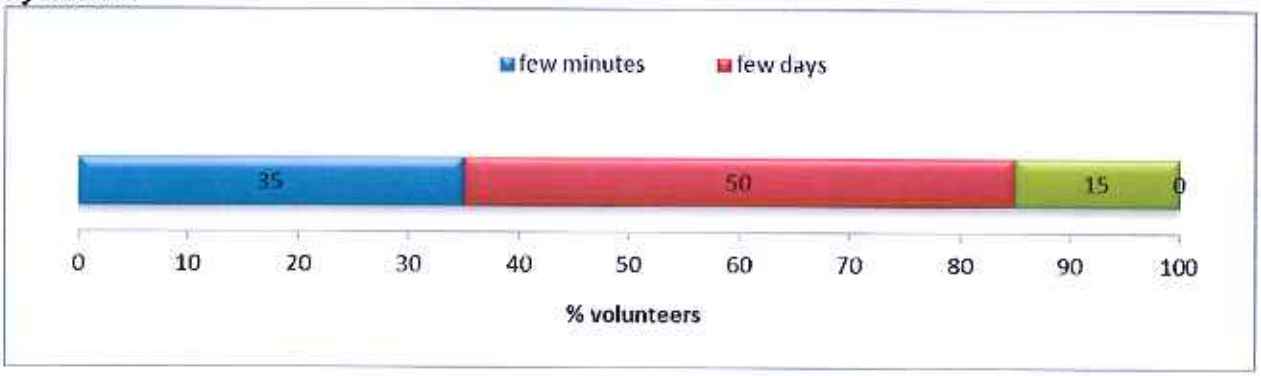
Questionnaire concerning a sensorial / psichorheological assessment relative to the tested product

To obtain a judgement from potential customers on product performances, the 20 subjects who took part to the study answered to a questionnaire on a subjective evaluation of the tested product.
Here below are reported all questions of the questionnaire and their answers are represented in the form of graphs. For the graphical representation of the multiple choice answers of each question, the percentage of volunteers who expressed the same opinion was calculated.

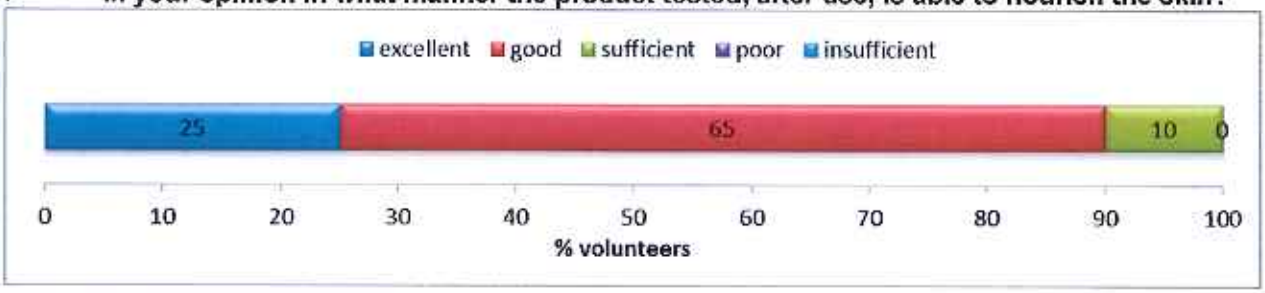
➢ **In your opinion in what manner the product used improves the following skin parameters?**



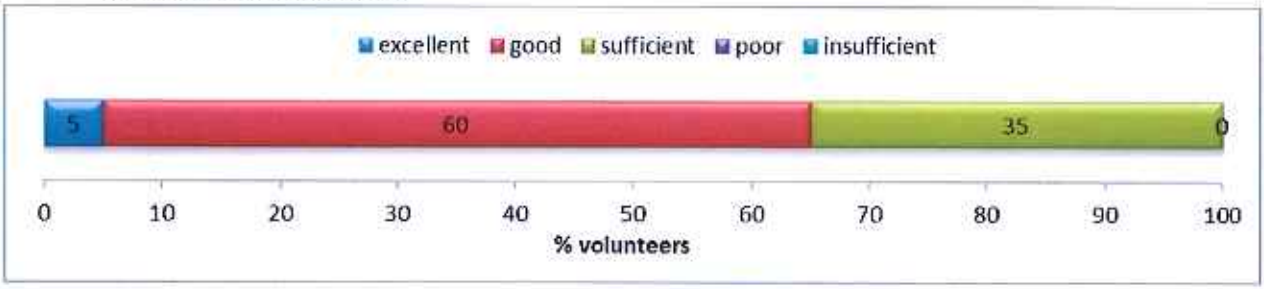
➢ **How long after the first application have you noticed an improvement in the skin hydration?**



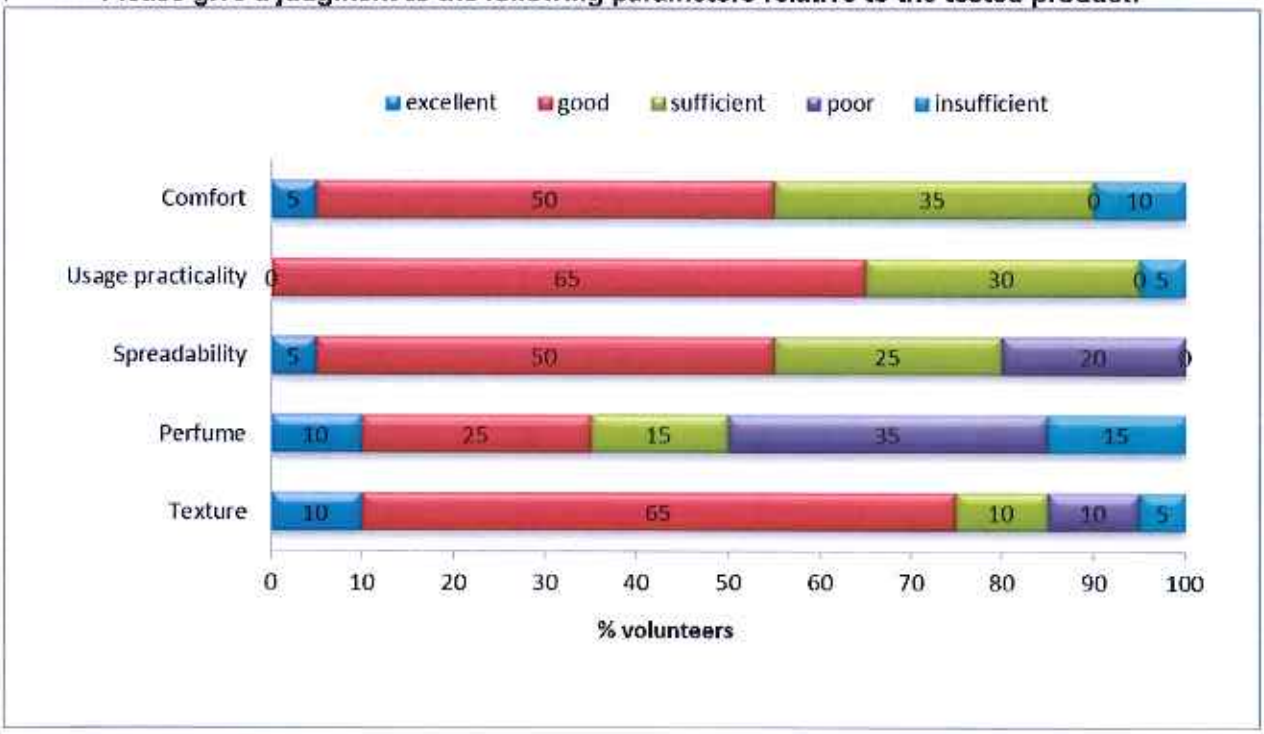
➢ **In your opinion in what manner the product tested, after use, is able to nourish the skin?**



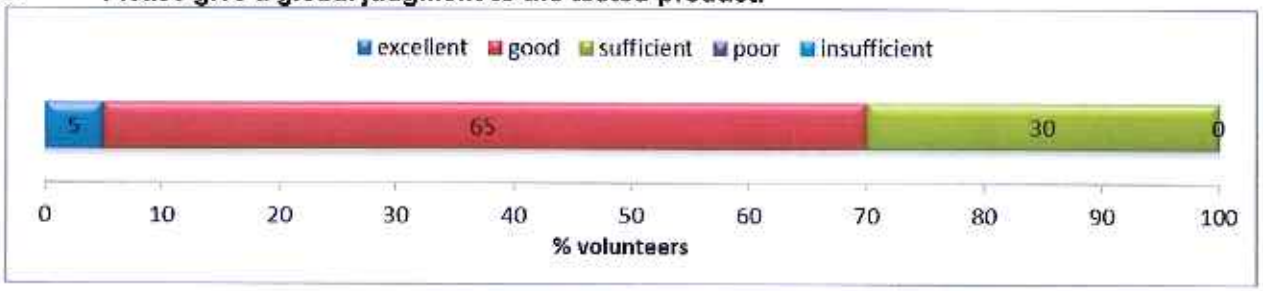
➢ **In your opinion in what manner the product tested, after use, is able to protect against sensation of tired skin?**



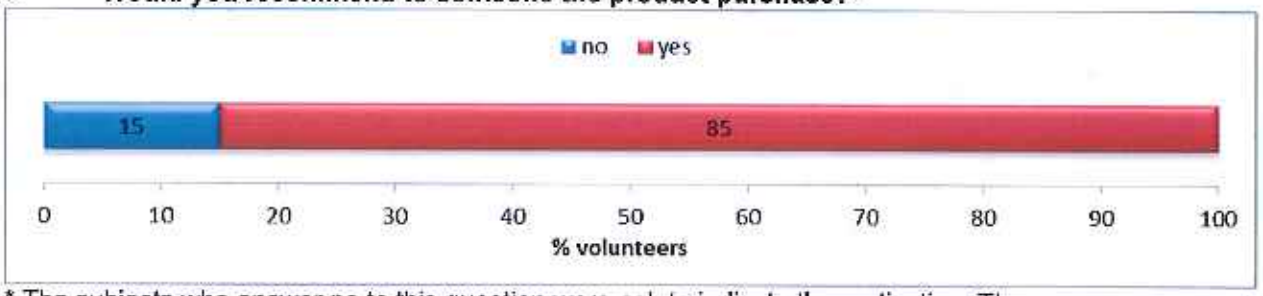
➢ **Please give a judgment to the following parameters relative to the tested product:**



➢ **Please give a global judgment to the tested product:**

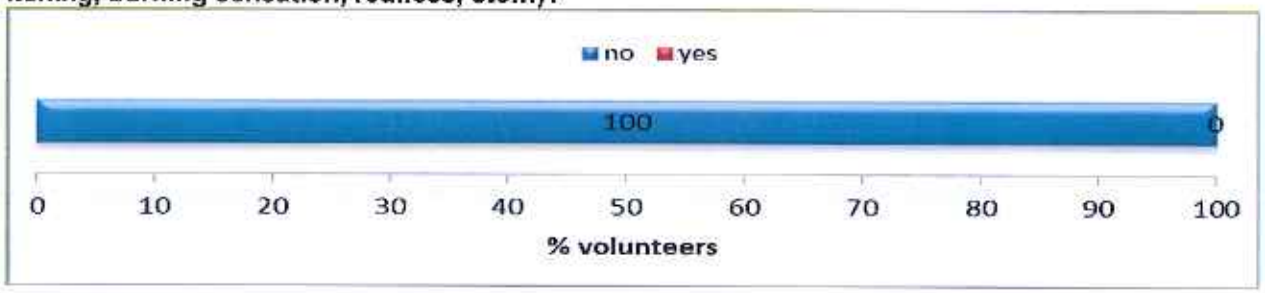


➢ **Would you recommend to someone the product purchase?***



* The subjects who answer no to this question were ask to indicate the motivation. The answers were:
- The product leaves a yellowish color on the skin
- bad perfume
- the product leaves traces on clothes

➢ **After the product usage did you note adverse effect caused by the product itself (irritation, itching, burning sensation, redness, etc...)?**



ANNEX 4

Inci list

Aqua, Alcohol denat., Petrolatum, Paraffinum liquidum, Glyceryl stearate, Isopropyl myristate, Tocopheryl acetate, Stearyl alcohol, Cetyl alcohol, Polysorbate 80, Cinnamomum camphora, Cetearyl ethylhexanoate, Salicylic acid, Cianocobalamin, Glycin soja, Daucus carota sativa seed oil, Beta-carotene, Riboflavin, Pyridoxine HCl, Biotin, Formic acid, Thiamine nitrate.