ISPE
Institute of Skin and Product Evaluation

Milano, December 14th 2011

**CLINICAL, INSTRUMENTAL AND PHOTOGRAPHIC EVALUATION
OF THE EFFICACY OF A TOPICAL TREATMENT FOR ACNE LESIONS**

METHOD: Ref. E30U + E12U + S14C

CUSTOMER: **GALENIA BIOTECNOLOGIE srl
                       Via Strada del Casas, 6/3
                       10090 Rosta (TO)**

PRODUCTS: **KERALISE CREMA
                       KERALISE PURIFYING CREAM
                       Batch 2990, Code 018
                       Ref. ISPE: 317/11/01 – 691/11**

                       **KERAPIL GEL DETERGENTE
                       KERAPIL PURIFYING SCRUB
                       Batch 16060, Code 019
                       Ref. ISPE: 317/11/02 – 692/11**

STARTING DATE OF THE STUDY: 11/10/2011
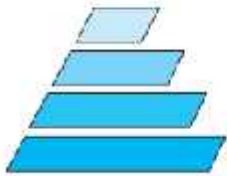
COMPLETION DATE: 13/12/2011

ETHICAL AND QUALITY CRITERIA
The current study was carried out in compliance with the quality assurance system requirements, according to the principles of good laboratory practice (GLP) and good clinical practice (GCP), as well as the principles established by the World Medical Association in the Declaration of Helsinki.

REFERENCES
The data given in this report are exclusively related to the tested sample.
This report can be only in full reproduced.

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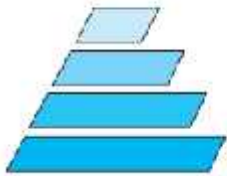Director of the Laboratory
Dr. Luigi Rigano

INDEX

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1. SAMPLES DATA SHEET

SAMPLES REF: **KERALISE CREMA**
KERALISE PURIFYING CREAM
Batch 2990, Code 018

Ref. ISPE: 317/11/01 – 691/11

KERAPIL GEL DETERGENTE
KERAPIL PURIFYING SCRUB
Batch 16060, Code 019
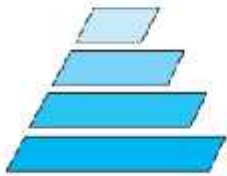
Ref. ISPE: 317/11/02 – 692/11

SAMPLES ARRIVAL DATE: 03/10/2011

PRODUCTS:

- PHYSICAL FORM: cream/gel
- COLOUR: white/colourless with granules

FORMULAE:

- KNOWN /yes/
- OTHER INFORMATION / /

OTHER INFORMATION RELATED TO THE PRODUCTS SAFETY:
None

FILE: FILE: 1 sample with the code number **Ref. ISPE: 317/11/01 – 691/11**, 1 sample with the code number **Ref. ISPE: 317/11/02 – 692/11** and the study findings will be kept filed in our archives for one year and for ten years respectively. After these periods, the samples and the findings report will be discarded, unless otherwise required by the client.

**CLINCAL, INSTRUMENTAL AND PHOTOGRAPHIC EVALUATION
OF THE EFFICACY OF A TOPICAL TREATMENT FOR ACNE LESIONS
(Ref. E30U + E12U + S14C)**

2. AIM OF THE STUDY

The aim of the study is to assess the efficacy of a treatment constituted by a cleansing gel and a cream in normalizing the level of sebum and in reducing the acne lesions of the face.

The efficacy of the treatment is shown by a statistically significant decrease in the following parameters:

- basal time values of skin sebometry;
- number of acne lesions (comedones, papules and pustules);
- acne intensity score.

3. SELECTION OF VOLUNTEERS

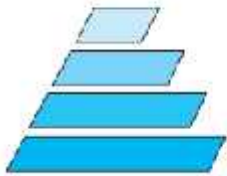### 3.a Criteria for recruitment and admission

The test is carried out according to the Declaration of Helsinki on 20 volunteers, average age 26 years.

Subjects are informed of the nature, purpose and risk of the study and give their written consent before participating in the test.

The selection is carried out according to the following criteria:

3.b Inclusion Criteria

- Race Caucasian.
- Male and female subjects, 18 - 45 years old, in general good health.
- Subjects suffering from acne on the face (grade I and II of the Leeds revised acne grading system – Annex I).
- Subjects able to follow all study directions and to commit to all follow-up visits for the duration of the study.
- Subjects who complete the informed consent process.
- Subjects who avoid the exposure to UV radiation and the use of tanning beds for the duration of the study.

3.c Exclusion criteria

- Pregnant or nursing females.
-Subjects with a history of unusual skin reactions to skin care toiletry products, cosmetics, or sensitivity to any of the test article components.
- Subjects who are taking topical or systemic drugs that could affect the results of the test (anti-inflammatory agents, corticosteroids, etc).
- Subjects showing systemic diseases or skin disorders (such as eczema, psoriasis, severe acne, etc.) that may affect the evaluation of the test articles or increase risk to the subject.
- Subjects currently using treatments (either topical and/or systemic) for the reduction of acne lesions.

3.d Drop - out

The following reasons are considered sufficient cause for interrupting the subject's participation in the study:
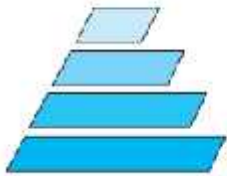
- o free choice of the subject;
- o reasons not correlated with the treatment (e.g., onset of disease, surgical operation);
- o reasons correlated with the treatment (e.g., irritant or allergic reactions).

Details of any case of drop-out are anyway included.

3.e Restrictions

During the study, subjects are instructed not to apply the products on any other site than the prescribed one.

For the whole duration of the test, the subjects should not use different products on the tested area (apart from make-up products) and should avoid UV rays exposure.

4. INSTRUMENTS

4.a. Sebumeter SM 815, Courage & Khazaka

The device is fitted with a manual counter featuring a ribbon 0.1 cm wide and with a surface contact area of 64 mm^2, used to absorb the sebum from the skin.

After setting the counter to zero, the head of the device is held against the skin for 30 seconds and then inserted inside the optical device.
When the head carrying the ribbon surface which has soaked up the sebum is inserted in the device, a closed circuit is created which lights a beam projected towards the ribbon; this beam is reflected by a mirror onto a photocell and amplified by a microamperometer.

The greater the quantity of lipids present on the ribbon, the more transparent the film will become: the numerical values read on the display are directly proportional to the transparency of the ribbon and hence to the quantity of lipids present on it.
Sebometric values range from 50 to 300 and are expressed directly in micrograms/cm^2.

In the case of values higher than 300 or lower than 50, it is not possible to obtain a precise count of the quantity of sebum.
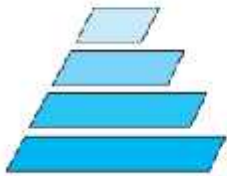
4.b. Digital Images, Fotofinder Dermoscope Ver.2.0

Fotofinder Dermoscope is a powerful and versatile system that allows carrying out and memorizing recorded images of any skin surface.

The system consists in a high-definition colour video camera which is able to magnify any surface on which it is placed by means of a series of magnifying lenses.

The digital images are shown on the screen in their real colours. This allows the observer to view the smallest details.

The software can also compare the recorded images to references recorded by the user.

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scritta nel'Albo ufficiale dei Laboratori di Ricerca altamente qualificati del Minist. dell'Università e della Ricerca (G.U.46/2002)

5. METHOD

5.a. Method of Evaluation

The test was carried out in a temperature and humidity-controlled room (24 ± 2 °C; 50 ± 10 %R.H.). The measurements of skin sebometry were performed after a 30-minute acclimatization.

Volunteers were asked to wash their face 3 hours before the instrumental measurements and not to apply any product on their face for at least 12 hours before each control time.

The treatment was used daily, in the morning and in the evening. The volunteers applied the cream after washing their face with the cleansing gel.

Instrumental evaluation
The **measures** of **skin sebum** were taken on the forehead, on the nose fold and on the chin.
Besides, **digital images** of each side of the face were taken (Annex II and cd-rom attached).

Clinical evaluation
A score was given to the level of seborrhoea, erythema and dryness/desquamation according to the following 4-point scale: **0** = absent; **1** = mild; **2** = moderate; **3** = severe.

Count of the acne lesions
A **count of the acne lesions** on the face of the selected volunteers was performed. The acne lesions can be divided by typology in the following way:
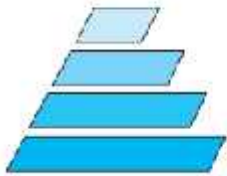
Non-inflammatory lesions:
Open comedones or blackheads: they are the result of the obstruction of the sebaceous follicles dilated by the hyperkeratinization and by the oxidation of the sebum within them.
Closed comedones or whiteheads: white or pink micro-papulose non-inflammatory lesions

Inflammatory lesions:
Papules: dark pink or red bumps, sometimes painful.
Pustules: white or yellow spots.

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Clinical Overall Assessment

A **score** was assigned to the acne intensity on the basis of the number of comedones, papules and pustules on the whole face according to the following scale:

1 = mild ($\leq$ 20 comedones and/or $\leq$ 6 papules/pustules)
2 = moderate (> 20 $\leq$ 40 comedones and/or >6 $\leq$ 16 papules/pustules)
3 = severe (> 40 comedones and/or > 16 papules/pustules).

After the basal evaluations, the treatment was given to the volunteers. The subjects came back to the laboratory to perform the final clinical and instrumental assessments after 4 weeks of use.

Subjective Evaluation

The volunteers expressed their opinion about the efficacy, the pleasantness and the tolerability of the treatment by means of a specific questionnaire on the following topics:

➢ efficacy in reducing acne lesions;
➢ efficacy in making skin less greasy;
➢ efficacy in making skin less shiny;
➢ soothing efficacy;
➢ purifying efficacy;
➢ exfoliant efficacy;
➢ moisturizing efficacy of the cream;
➢ efficacy in making skin smoother and softer;
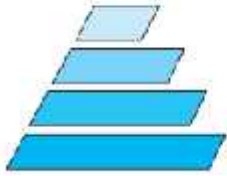➢ mildness of the treatment;
➢ adverse reactions.

For each question the volunteers expressed their opinion on a 4-point intensity scale.

Example:

Do you judge the treatment mild for the face skin?

▪ very much
▪ fairly
▪ not very much
▪ not at all

5.b. Mathematical elaboration

Evaluation of the skin sebum

Mean values, standard deviations, variations and percentage variations were calculated for each set of values.
Following the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test) the data were statistically compared by means of **Wilcoxon Test** for non-parametric data.

The groups of data were considered statistically different for a probability value **p≤0.05**.

### Clinical evaluations and count of the acne lesions

Mean values and standard deviations were calculated for each set of values.
Following the results of normality tests (Kolmogorov-Smirnov test, Lilliefors test and Shapiro-Wilk test) the data related to the number of comedones (open and closed) and of pustules, to the acne intensity score and to the clinical evaluations were statistically compared by means of **Wilcoxon Test** for non-parametric data; the data related to the number of papules were instead compared by means of **t-test** for dependent and parametric data.

The groups of data were considered statistically different for a probability value **p≤0.05**.

5.c. Reading of the questionnaire

For each level of intensity the number and the percentage of answers was reported in the tables.
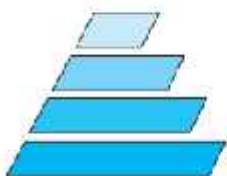
6. RESULTS: TABLES AND GRAPHS
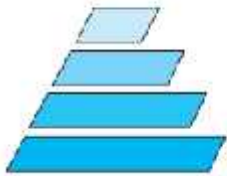
6.a. SKIN SEBOMETRY

A _statistically significant decrease_ in the skin sebometry values was detetcted at the end of the 4 weeks of treatment.

Table 1: mean values ($\mu g/cm^2$), standard deviations, variation, percentage variation and statistical comparison.

T_0	T_{4wks}	Variation $T_{4wks}- T_0$	% Variation	T_0 vs T_{4wks}
mean 106.9 std. dev. 27.8	mean 90.2 std. dev. 31.6	-16.7	-15.6%	p<0.01

Graph 1: the graph shows the trend of skin sebometry values registered at the beginning and at the end of the treatment.

SEBUM $\mu g/cm^2$
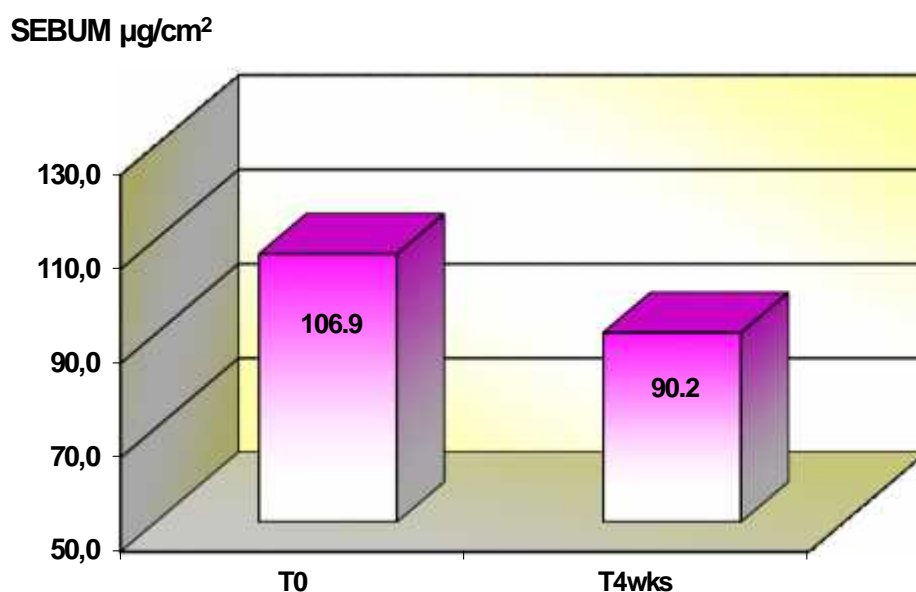
6.b. COUNT OF ACNE LESIONS (comedones, papules, pustules)

A statistically significant decrease in the number of coemdones, papules and pustules was evidenced after 4 weeks of treatment.

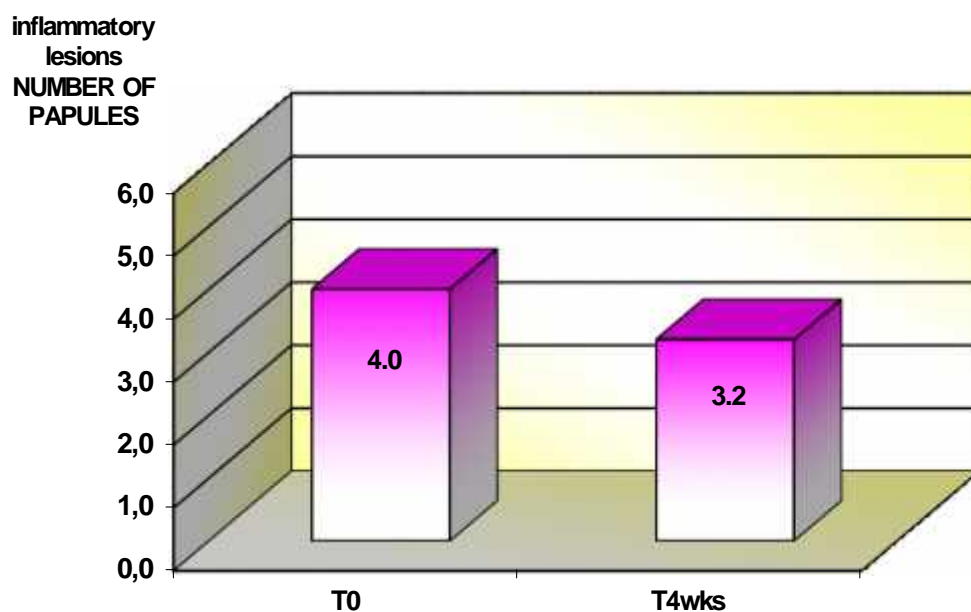**Table 2:** mean values and standard deviations of the number of acne lesions and statistical comparison.

	T_0	T_{4wks}	T_0 vs T_{4wks}
NUMBER OF COMEDONES (OPEN AND CLOSED)	14.9 ± 12.5	12.5 ± 11.8	**p=0.001**
NUMBER OF PAPULES	4.0 ± 2.4	3.2 ± 2.5	**p<0.05**
NUMBER OF PUSTULES	3.9 ± 3.1	2.6 ± 2.7	**p<0.01**

Graph 2: the graph shows the variation in the number of comedones at the beginning and at the end of the treatment.
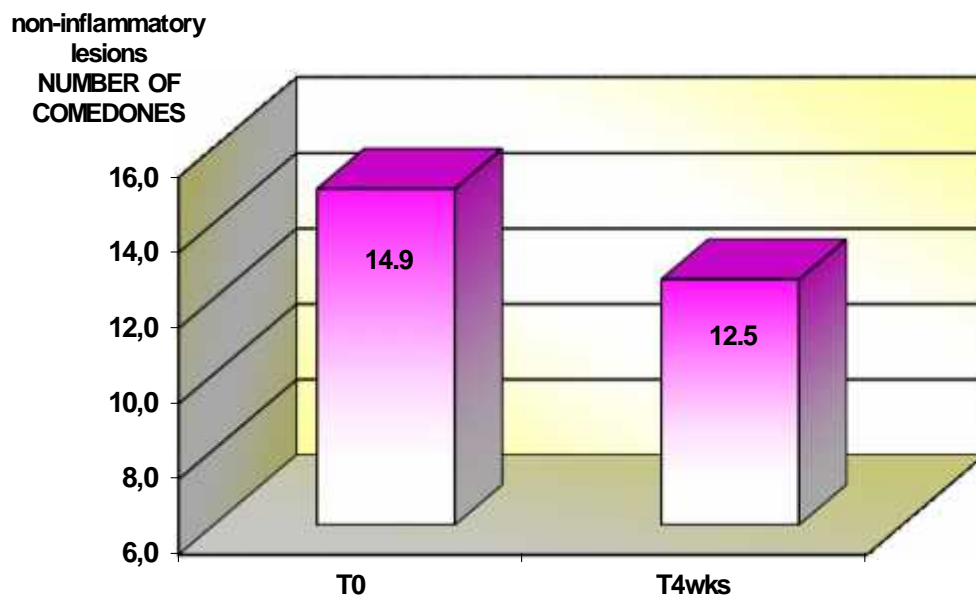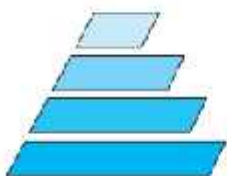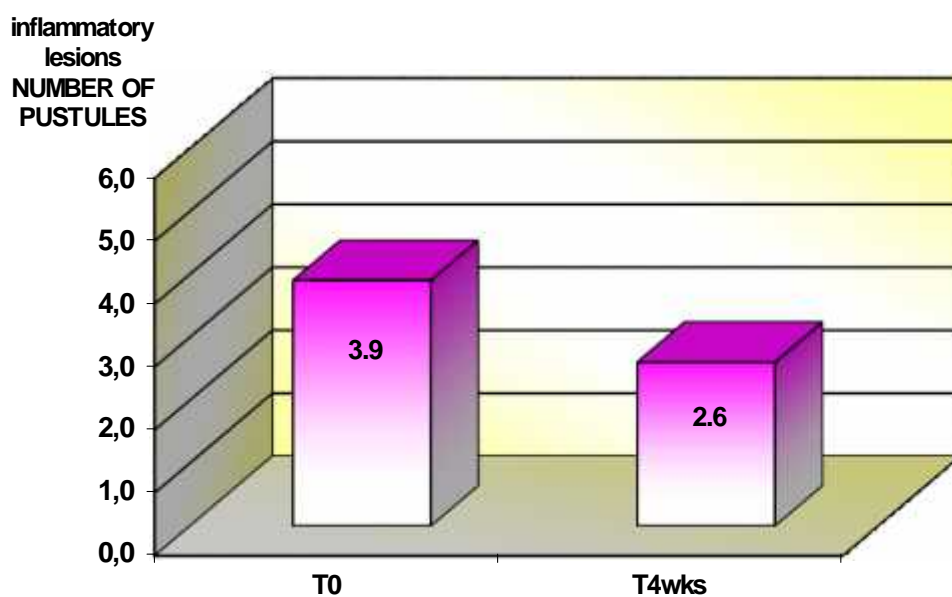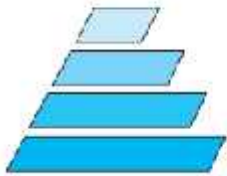
Graph 3: the graph shows the variation in the number of papules at the beginning and at the end of the treatment.

Graph 4: the graph shows the variation in the number of pustules at the beginning and at the end of the treatment.
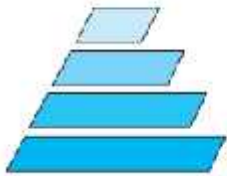
6.c. CLINICAL OVERALL ASSESSMENT

A score was assigned to the acne intensity on the basis of the number of comedones, papules and pustules according to the following scale:

1 = mild ($\leq$ 20 comedones and/or $\leq$ 6 papules/pustules)
2 = moderate (> 20 $\leq$ 40 comedones and/or >6 $\leq$ 16 papules/pustules)
3 = severe (> 40 comedones and/or > 16 papules/pustules)

Table 3: mean values and standard deviations of the assigned score and statistical comparison.

T_0	T_{4wks}	T_0 vs T_{4wks}
1.7 ± 0.6	1.5 ± 0.6	p>0.05

No statistically significant variations of the considered parameter were detected at the end of the treatment.
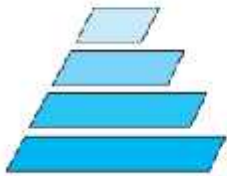
6.d. CLINICAL EVALUATION

The clinical evaluation of *seborrhoea, erythema* and *dryness/desquamation* was performed according to the following 4 point scale: **0** = absent; **1** = mild; **2** = moderate; **3** = severe.

Table 4: mean values and standard deviations of the assigned scores and statistical comparison.

	T_0	T_{4wks}	T_0 vs T_{4wks}
SEBORRHOEA	1.6 ± 0.7	1.4 ± 0.6	p>0.05
ERYTHEMA	0.7 ± 0.7	0.7 ± 0.7	p>0.05
DRYNESS DESQUAMATION	0.1 ± 0.2	0.1 ± 0.2	---

The statistical analysis was not performed on the data related to dryness/desquamation because they did not change at the end of the test.

No statistically significant variation in the considered parameters was detected at the end of the treatment.

7. QUESTIONNAIRE'S RESULTS

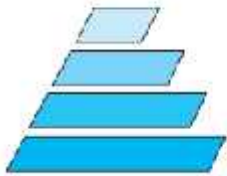### 1) Do you judge the treatment effective in reducing acne lesions?

	Number of answers	%
Very effective	3	15%
Fairly effective	12	60%
Not very effective	3	15%
Not effective at all	2	10%

2) Do you judge the treatment effective in making skin less greasy?

	Number of answers	%
Very effective	8	40%
Fairly effective	8	40%
Not very effective	3	15%
Not effective at all	1	5%

3) Do you judge the treatment effective in making skin less shiny?

	Number of answers	%
Very effective	4	20%
Fairly effective	10	50%
Not very effective	6	30%
Not effective at all	0	0%

4) Do you judge the treatment effective in soothing the face skin?

	Number of answers	%
Very effective	5	25%
Fairly effective	10	50%
Not very effective	2	10%
Not effective at all	3	15%

5) How effective do you judge the treatment as regards its purifying effect?

	Number of answers	%
Very effective	5	25%
Fairly effective	11	55%
Not very effective	3	15%
Not effective at all	1	5%

6) How effective do you judge the treatment as regards its exfoliant efficacy?

	Number of answers	%
Very effective	10	50%
Fairly effective	8	40%
Not very effective	2	10%
Not effective at all	0	0%

7) Do you judge the cream effective in moisturizing the skin?

	Number of answers	%
Very effective	4	20%
Fairly effective	11	55%
Not very effective	4	20%
Not effective at all	1	5%
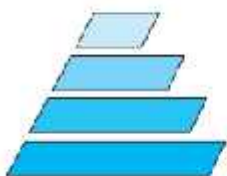
8) Do you judge the treatment effective in making the skin smoother and softer?

	Number of answers	%
Very effective	5	25%
Fairly effective	12	60%
Not very effective	3	15%
Not effective at all	0	0%

9) Do you judge the treatment mild for the face skin?

	Number of answers	%
Very much	5	25%
Fairly	8	40%
Not very much	5	25%
Not at all	2	10%

10) Did you perceive any adverse reaction during the application of the treatment (erythema, burning sensation, uncomfortable sensation)?

	Number of answers	%
Molto intense	0	0%
Fairly intense	1	5%**
Not very intense	1	5%*
Absent	18	90%

* The subject nr 6 perceived a slight burning and noticed a mild redness on the face skin soon after the use of the treatment, especially in the first days of application.

** The subject nr 2 perceived a fairly intense burning and redness on the face skin soon after the application of the treatment. Also in this case the symptoms were perceived above all in the first period of use of the treatment.
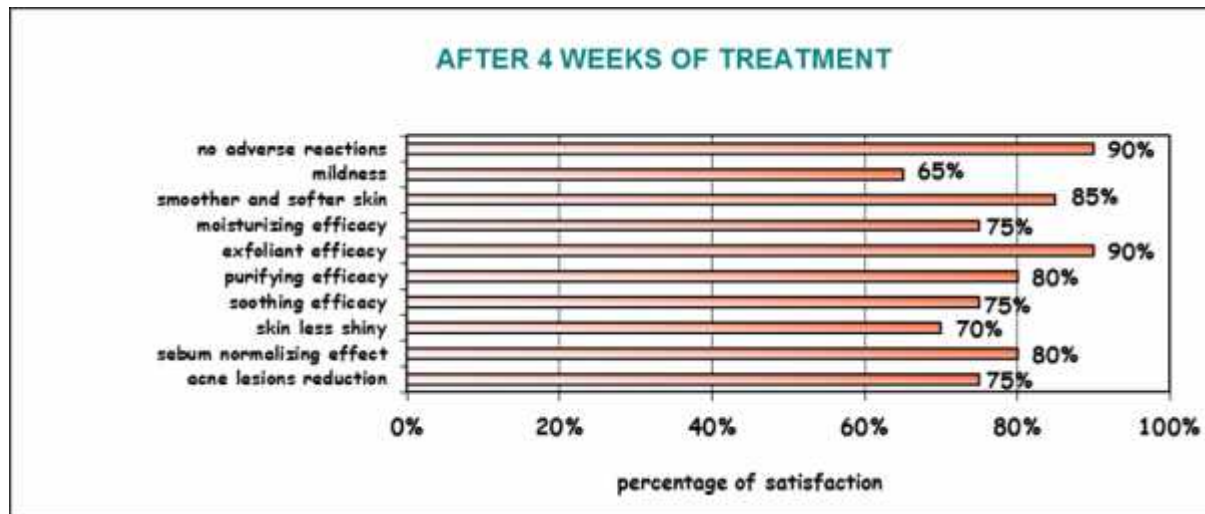
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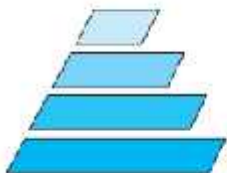
8. CONCLUSIONS

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In order to evaluate the efficacy of the treatment constituted by **KERALISE CREMA, KERALISE PURIFYING CREAM, batch 2990, code 018, Ref. ISPE: 317/11/01 – 691/11** and **KERAPIL GEL DETERGENTE, KERAPIL PURIFYING SCRUB, batch 16060, code 019, Ref. ISPE: 317/11/02 – 692/11** in normalizing the face skin and in reducing acne lesions, 20 volunteers suffering from acne (grade I and II of the Leeds revised acne grading system) used it for 4 weeks.

The following results have been obtained after 4 weeks of treatment:

- a statistically significant decrease in the skin sebometry values;
- a statistically significant decrease in the number of acne lesions (comedones, papules and pustules) on the face.

The following graph describes the results of the subjective evaluation.

AFTER 4 WEEKS OF TREATMENT

Category	Percentage
no adverse reactions	90%
mildness	65%
smoother and softer skin	85%
moisturizing efficacy	75%
exfoliant efficacy	90%
purifying efficacy	80%
soothing efficacy	75%
skin less shiny	70%
sebum normalizing effect	80%
acne lesions reduction	75%

percentage of satisfaction

Responsabile for the evaluation
Dr. Claudia Cartigliani

Responsabile for the laboratory
Dr. Adriana Bonfigli

Dermatologist
Dr. Fernanda Distante

9. BIBLIOGRAPHY

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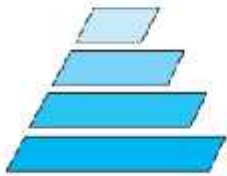
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LEEDS REVISED ACNE GRADING SYSTEM

Grade I

Grade II

Grade III

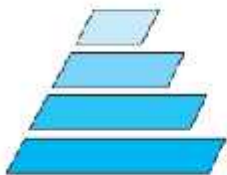**Grade IV**

Grade V

Grade VI

Grade VII

Grade VIII

Grade IX

Grade X

ANNEX II

DIGITAL IMAGES

A selection of the digital images taken at the beginning and at the end of the study is reported below. All the digital images can be found in the cd-rom attached to the report.
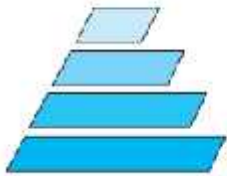
SUBJECT 2

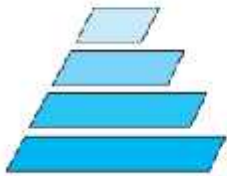**BASAL TIME**

AFTER 4 WEEKS OF TREATMENT

ANNEX II

SUBJECT 3

BASAL TIME

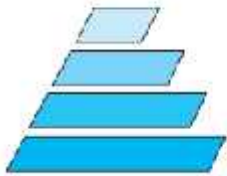**AFTER 4 WEEKS OF TREATMENT**

ANNEX II

SUBJECT 5

BASAL TIME

AFTER 4 WEEKS OF TREATMENT

ANNEX II

SUBJECT 12

BASAL TIME

AFTER 4 WEEKS OF TREATMENT