EVALUATION OF THE EFFECTIVENESS OF STRETCH MARKS COSMETIC PRODUCTS

Report number: S/10/1850

Test product: **Elicina**

Customer: Unipro Servizi s.r.l.

              Via Accademia, 33

               20131 Milano

Sponsor: Bioelisir S.r.l.

              Via F.lli Kennedy, 40/b

              07041 Alghero (SS)

Aim of the study

The study aims to investigate the effectiveness of the cosmetic formulation *Elicina* in the treatment of stretch marks by a 2 months "in use" test.

Experimental protocol

The study has been carried out according to Helsinki declaration (Ethical Principles for Medical Research Involving Human Subjects).

Twenty healthy volunteers, of both sex, between 22 and 38 years old, were selected according to the following inclusion criteria:

- good general health;
- absence of cutaneous diseases;
- people who has stretch marks;
- people who does not show pigmentary lesions or other lesions on the interest area that could interfere with the study evaluation;

- people who does not present history of hypersensitivity to the common components in cosmetic formulations;
- women not being pregnant or breastfeeding;
- people who agreed not to use other skin products for the duration of the test in the tested areas;
- subjects who signed the consent form (see Annex I)

and following exclusion criteria:

- subjects that didn't meet the inclusion criteria above;
- some drug treatment that could interfere with the assessment of the study;
- participation in similar study by at least 60 days;
- subjects with allergies to some components of the product.

Drop out

During the study some case of drop out may occur. Possible causes of interruption of the test are:

- inability to contact the volunteer;
- free choice of volunteer;
- health reasons related to the treatment in study (i.e., onset of reaction of intolerance towards the product)

Length of Study

The study involves two months of treatment

Design of the study

The product is applied daily by massage until completely absorbtion over one body area.

The controlateral area with stretch marks is treated with a "placebo" formulation without active, as a control.

Instrumental evaluation

At the beginning of the trial, after 1 month and after 2 months of the product application the following instrumental image evaluation is carried out.

All measurements were made in an air conditioned room with controlled temperature and humidity (T 22°C, r.h. 50 ± 5%); subjects were preconditioned for at least 15 minutes before the measurements.

These are techniques involving the contact between the skin and a series of probes free of discomfort, pain or individual damage.

<u>Image evaluation</u>

The image evaluation was performed by the apparatus PRIMOS[pico] (GFM,Teltow, Germany), an optical analysis system , which can capture the 3D image of the skin surface.

The 3D image acquired with the instrument can be transformed by the software Primos 5.7 to obtain a colored image in which each shade of color (from blue to yellow) is related to a height, as shown below:

Height (mm)

-2   -1   0   1   2

Thus, the presence of a groove will be indicated by a color gradient that can go from blue to green according to its depth.

Parameters characterizing skin microreliefs can be measured at the glance thanks to this three-dimensional topographic representation of the skin.

In particular, the parameter considered in this study is **Ra,** the average skin roughness**,** which is the integral of the function describing the skin profile curve and it corresponds to the curve area included above and below the average line (Figure 1).

This parameter is based on the sampling of all the points characterizing the profile, so it represents a true average because it is very significant in regard to the roughness of the skin.
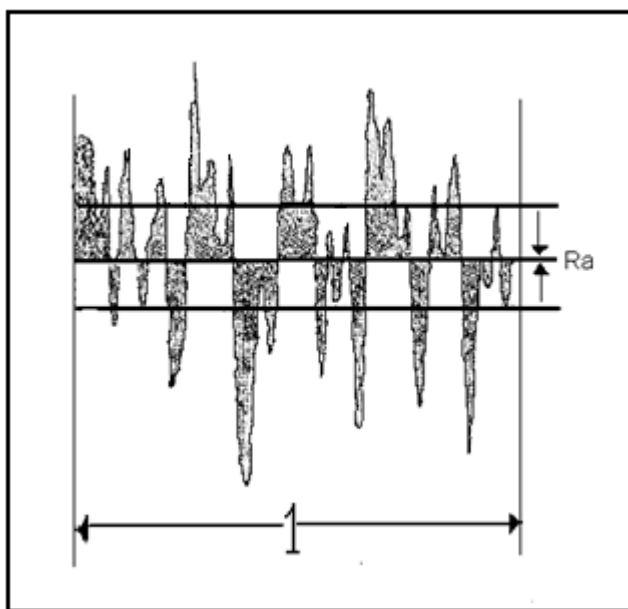
Fig.1 Roughness mean value (Ra)

The Ra is two-dimensional parameter. For skin with very anisotropic characteristics, the value of this parameter can be very different depending on the direction along which the profile being analyzed (Figure 2). Ra has a higher value in perpendicular orientation to respect to the grooves (X direction in the figure 2); in fact, $Rax > Ray$.

It must, therefore, to process simultaneously a large number of profiles that follow all possible directions and calculate the average of several measurements obtained.

The number of lines to be measured is an important factor; it is therefore very important to try as many lines as possible, to encompass the entire area of the captured image in the analysis; the higher the number of available profiles, the greater will be the precision of measurement.
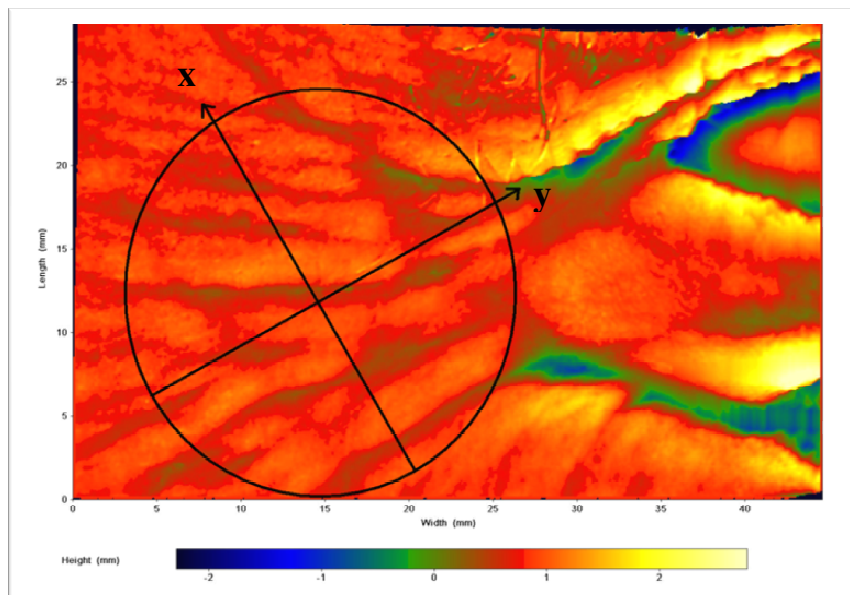

Fig.2 Microrelief image. In particular directions along which the profile being analyzed

With the PRIMOS[pico] system up to 32 radial lines are processed in a few seconds (Figure 3). The profiles must always be of the same size; in fact, a change in the length of the profile automatically lead to a difference in the values of the roughness parameter, Ra.

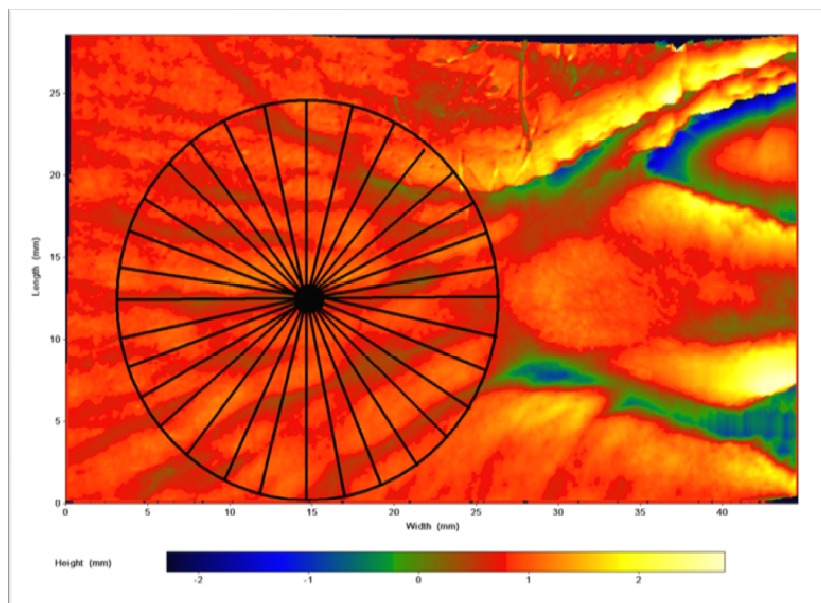**Fig.3** Microrelief image. Area analyzed by splitting up into 32 profiles
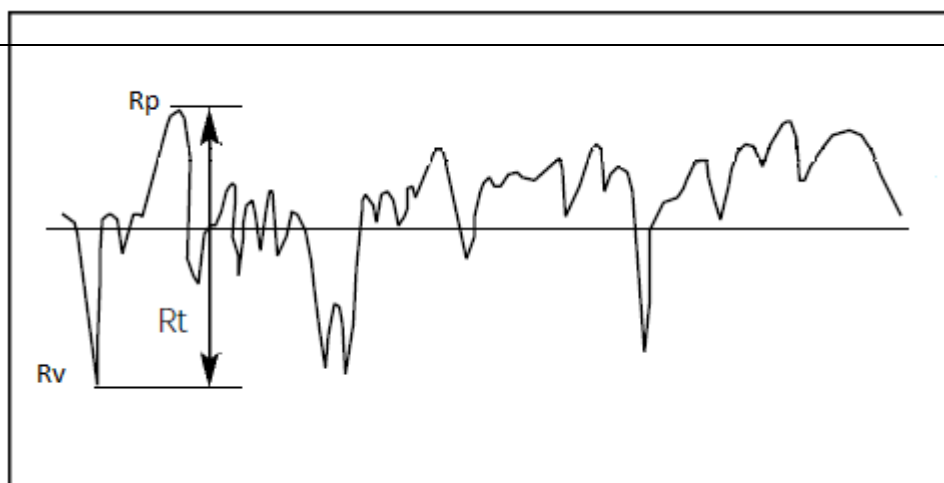

Fig.4 Components and higher value of Rt

Parameter Rt is the sum of the peak value (Rp) and the valley peak (Rv) parameters.

<u>Subjective evaluation</u>

The volunteers, at the end of the study, express an opinion regarding the subjective pleasantness, the cosmetic product effectiveness and tolerability using a questionnaire (Annex II).

Formulation INCI

Helix aspersa muller extract, paraffinum liquidum, propylene glycol, stearic acid, cetyl alcohol, sodium lauril sulfate, diazolidinyl urea, methylparaben, propylparaben, triethanolamine.

Statistical analysis

The study results are expressed as mean ± S.D. On the data collected statistical analyses with the tests for non parametric data and on repeated measures were carried out. A significance level of 5% was chosen, so the changes were considered statistically significant for $p < 0.05$.

Confidentiality

The information contained in this document and any additional information supplied contains commercially sensitive information that is privileged and confidential and may not be disclosed unless such disclosure is required by law or regulations. Persons to whom information is disclosed must also be informed that the information is privileged or confidential and may not be further disclosed by them.

All documentation generated during the course of the study will be treated as strictly confidential.

Publication

The results of this study may be published externally only with written permission of the sponsor.

All information concerning the study products supplied by the sponsor and not previously published is considered confidential and shall remain the sole property of the sponsor. The investigator agrees to use this information only in relation to this study and will not use it for other purposes without written consent from the sponsor.

It is understood by the investigator that the information from the study will be used by the sponsor in connection with the development of the study products and, therefore, may be disclosed as required to other investigators or to government agencies. It is understood that there is an obligation to provide the sponsor with complete test results and all data developed in the study.

Investigators

The Study Director was Dr. Paola Perugini, PhD, Pharmacist, Professor of Cosmetic Products at the Faculty of Pharmacy at the University of Pavia; the investigator was Dr. Mariella Bleve, degree in Pharmaceutical Chemistry and Technology, Pharmacist.

The study was carried out at the Department of Pharmaceutical Chemistry of The Faculty of Pharmacy, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy.

Results and discussion

The aim of this study was to evaluate the effect of the cosmetic product *Elicina* in the treatment of stretch marks.

To this end, the product was applied on specific body area and a placebo on the specific other one for two months. The evaluation of deep of cutaneous drop in the area with stretch marks was carried out before application of the product, after 1 month and at the end of the trial.

All subjects completed the trial and no cases of intolerance to the product were detected. The Primos system has allowed us to evaluate, by matching, the same skin area in the three subsequent analysis times. For example, figures 5-6 show stretch marks in color and black and white images obtained at 0 and 2 months time and their elaborations by the software. The images concern the area treated with *Elicina.*

Fig.5: Images of stretch marks and their elaboration in color and black and white images obtained at 0 and 2 months time. The images concern the area treated with *Elicina.* The arrow indicates the stretch marks that are not visible after 2 months of treatment.
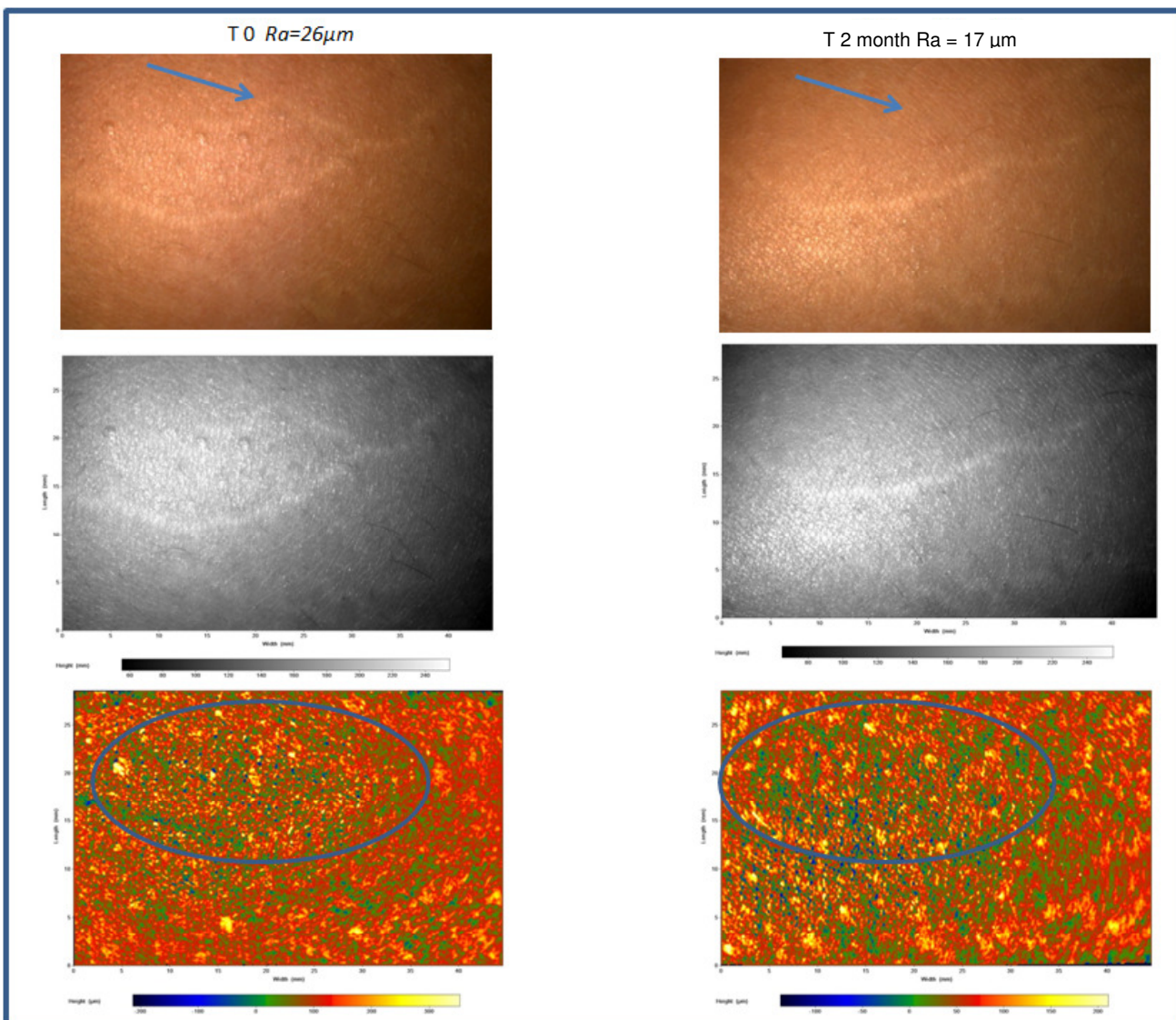
Fig.6: Images of stretch marks and their elaboration in color and black and white images obtained at 0 and 2 months time. The images concern the area treated with *Elicina*. The arrow indicates the stetch marks that are not visible after 2 months of treatment.
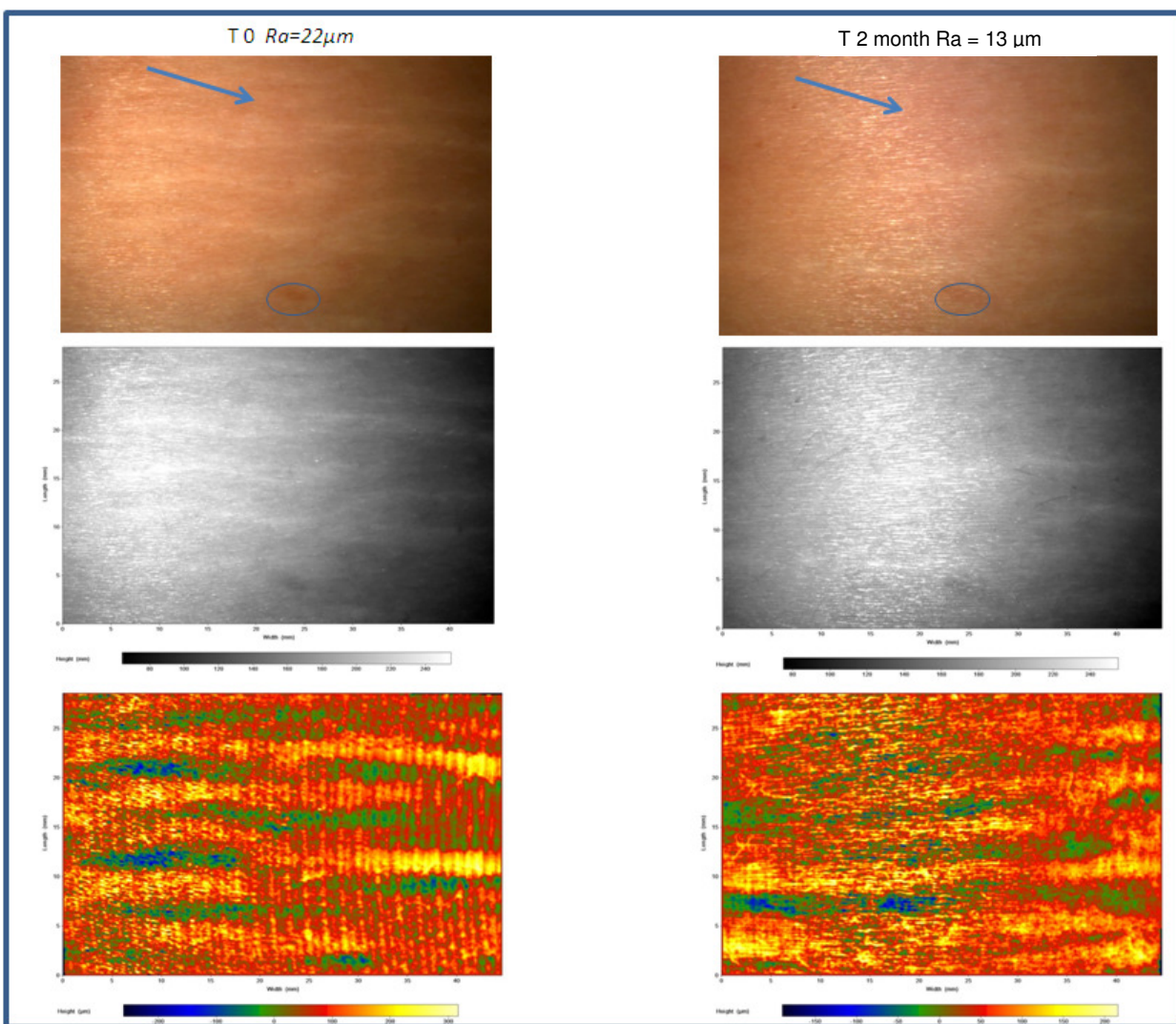
The parameter Ra is generically called roughness parameter and it is expressed in micrometers. This parameter is indicative of the depth of the cutaneous drop like stretch marks or wrinkles. The parameter Ra was obtained by processing 32 radial lines in order to obtain a measure of the average roughness correlated to sensory perception.

The results obtained are reported in the Tables 1 and 2.

Tab.1 Ra values (µm) of areas with stretch marks treated with *Elicina*		
Volunteer	**Ra t0**	**Ra t1**	**Ra t2**
Vol1	23	18	19
Vol2	22	15	21
Vol3	19	18	16
Vol4	22	13	13
Vol5	17	14	15
Vol6	26	18	17
Vol7	14	15	14
Vol8	24	22	24
Vol9	19	17	19
Vol10	17	13	14
Vol11	20	34	25
Vol12	18	18	14
Vol13	33	32	28
Vol14	24	19	18
Vol15	28	16	15
Vol16	20	16	15
Vol17	25	20	20
Vol18	19	19	20
Vol19	16	17	16
Vol20	18	22	19
Mean	21.1	18.8	18.2
S.D.	4.56	5.48	4.06
p	0.0071**		

Tab.2 Ra values (µm) of areas with stretch marks treated with placebo		
Volunteer	Ra t0	Ra t1 mes	Ra t2 mes
Vol1	24	21	21
Vol2	20	16	19
Vol3	15	16	16
Vol4	16	15	16
Vol5	20	15	16
Vol6	15	17	17
Vol7	21	17	22
Vol8	25	25	27
Vol9	18	17	16
Vol10	20	22	19
Vol11	33	31	30
Vol12	16	15	16
Vol13	23	-	24
Vol14	27	24	24
Vol15	25	21	21
Vol16	16	15	19
Vol17	18	19	19
Vol18	21	16	17
Vol19	16	17	17
Vol20	18	19	17
Media	20.35	18.84	19.65
D.S.	4.69	4.27	4
p		0.0332*	

The results show a decrease of 14.62% of Ra parameter in the areas treated with the product *Elicina,* and this reduction is statistically significant using the ANOVA test for repeated measures. The reduction of 3.44% found in the placebo area is significant but it is smaller than value found for the treated area.

In this study, the reduction in skin roughness is due to the smoothing action that the product have been in relation to the more superficial stretch marks. For this reason it was also investigated the parameter Rt, indicative of the maximum height of the same profile. The Tables 3 and 4 show the results for this parameter.

Tab.3 Rt values of areas with stretch marks treated with *Elicina*		
Voluntee	**Rt t0**	**Rt t1**	**Ratt2**
Vol1	179	124	130
Vol2	184	129	181
Vol3	153	139	140
Vol4	148	104	140
Vol5	126	103	115
Vol6	254	133	143
Vol7	123	114	92
Vol8	183	164	168
Vol9	148	145	151
Vol10	141	101	109
Vol11	151	277	183
Vol12	152	143	113
Vol13	240	238	201
Vol14	183	147	128
Vol15	233	124	107
Vol16	149	121	106
Vol17	178	151	152
Vol18	156	147	157
Vol19	121	132	125
Vol20	138	164	141
Mean	167	145	139.1
S.D.	37.98	43.07	28.94
p		0.0087**	

Tab.4 Rt values of areas with stretch marks treated with placebo		
Voluntee	**Rt t0**	**Rt t1**	**Rt t2**
Vol1	177	156	161
Vol2	157	119	152
Vol3	112	120	116
Vol4	117	140	137
Vol5	144	120	115
Vol6	120	131	123
Vol7	174	137	181
Vol8	187	186	205
Vol9	140	137	114
Vol10	165	161	150
Vol11	243	218	219
Vol12	131	107	119
Vol14	205	177	201
Vol15	200	170	164
Vol16	126	117	132
Vol17	129	165	125
Vol18	162	145	143
Vol19	132	134	151
Vol20	136	163	125
Mean	157.45	147.53	149.7
S.D.	35.08	28.15	31.58
p		ns	

The results show a decrease of 16.71% of Rt parameter in the areas treated with the product Elicina, and this reduction is statistically significant. On the contrary in the area treated with placebo there is not statistically significant change.
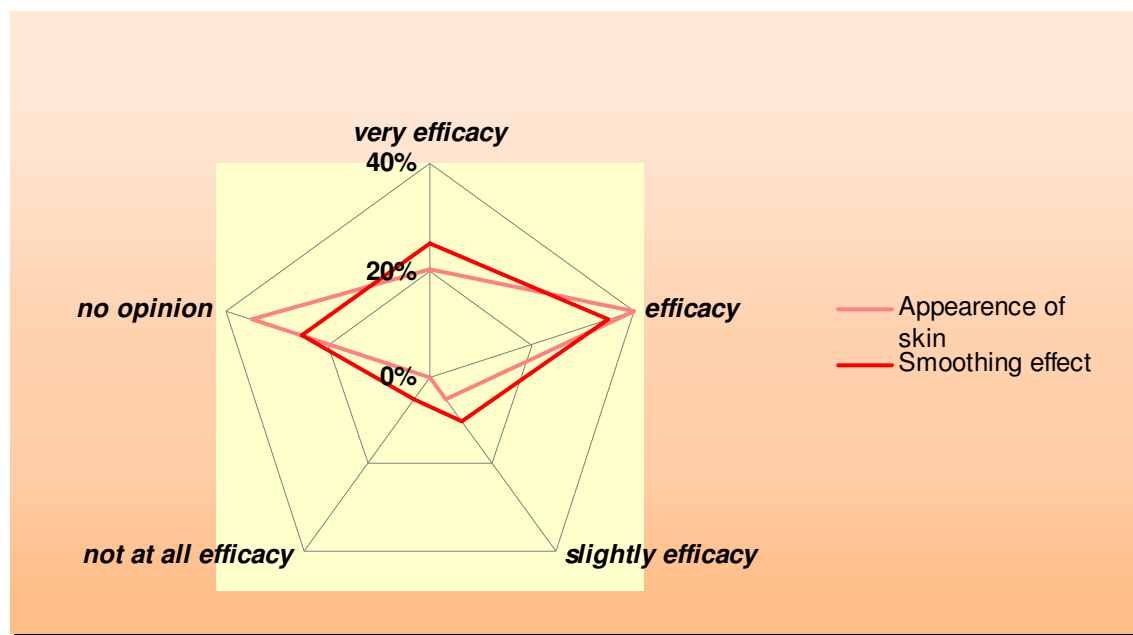
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Subjective assessment

The subjective evaluation of effectiveness, pleasantness and tolerability of *Elicina*, was carried out through questionnaires completed by volunteers at the end of the study.

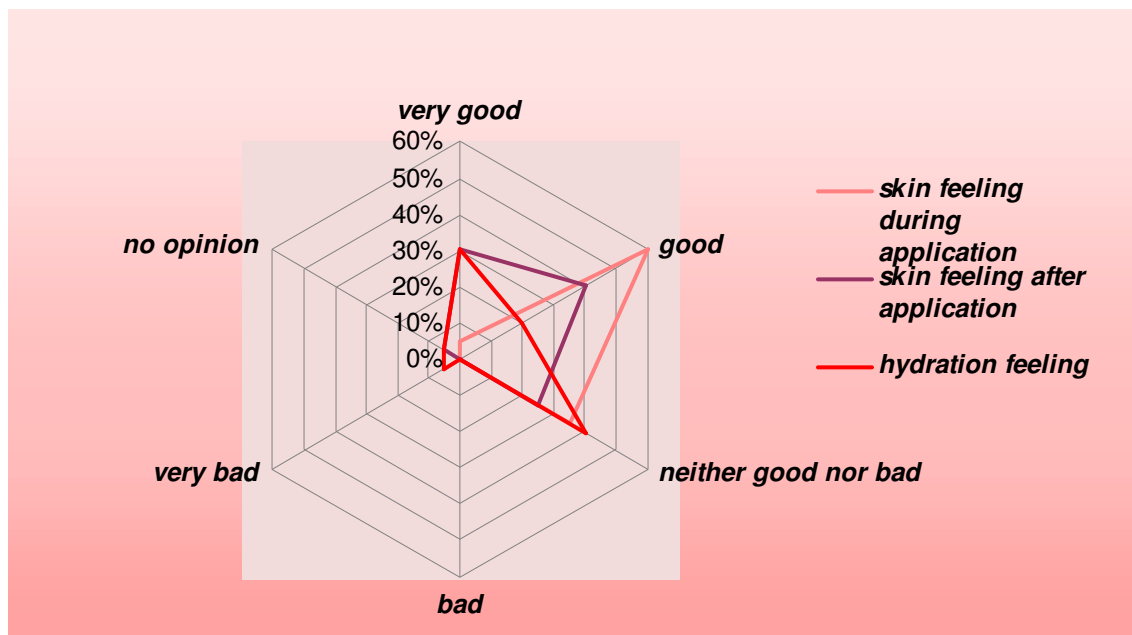All the subjects tolerated well or very well the product.

In the following graphs are reported the results of the subjective evaluation.

Improvement of the skin appearance and smoothing effect
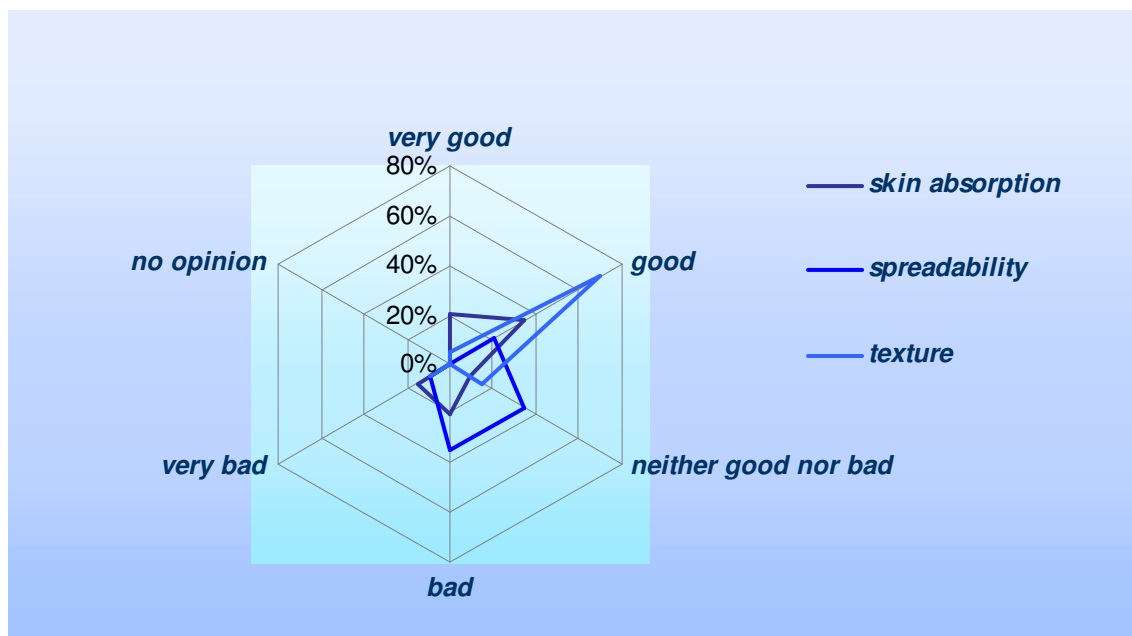
Sensation on the skin during and after application and hydration feeling

Product characteristics

About 80% of subjects are satisfied or very satisfied with the product and more than 60% would buy the product.

Conclusions

This study was intended to evaluate the tolerability and the efficacy of cosmetic product *Elicina* in the treatment of stretch marks blemishes.

At the end of this evaluation it is possible to affirm that:

➢ The continued daily use for two months of *Elicina* product showed no problems of skin tolerability; therefore the product *Elicina* is very well tolerated;

➢ The continued daily use for two months of *Elicina* product has resulted in a statistically significant reduction of the depth of stretch marks, this reduction is of about 15% after two months of treatment.

Date and signature: 22nd December 2010

_____
       Dr. Paola Perugini, Study Director

Annex I

DECLARATION OF CONSENT

The undersigned, ..., Born in ... ... on ... ...
residing at ... ...
States

To submit voluntarily to the study
Evaluation of efficacy of a cosmetic antiwrinkles product

Further states:
- To have been made aware of the usefulness of the test and the possible risks related to it;
- For not having taken part in similar tests over the past two months.

Pavia, ... By Faith ... ...

WRITTEN INFORMATION (PRIVACY)
We inform, within the meaning of 'Article 10 of Law 31 Dec. 1996, n.675 (Privacy Policy informatics) that the personal information you provide will be processed in compliance with the legislation mentioned above and the obligations of confidentiality imposed by the Act. The data collected will be used for purposes related to or useful for us to be carried out, using instruments that guarantee security and confidentiality and the treatment will be carried out with instruments that are not computerized. We inform you that the personal information you provide will not be covered nor communication, nor to spread and will be stored at our office for the time prescribed by law. Providing your personal information is an act of a voluntary nature and your refusal will not have any consequences. In relation to the data subject, you can still exercise their rights under Art. 13 of Law No. 675/1996.
CONSENT TO THE PROCESSING OF PERSONAL DATA GENERAL AND SENSITIVE.
Having understood the above, I agree, in accordance with Articles 11 and 12 of Law No 31 Dec. 1996 675, to process my personal data by the persons specified in the said information and in compliance with the same.

SIGNATURE

DIPARTIMENTO DI CHIMICA FARMACEUTICA

UNIVERSITA' DEGLI STUDI DI PAVIA

Segreteria amministrativa

Allegato II ANNEX II
Name: **Vol-No.:**
Study-No.: **Date:**

Questionnaire: Subjective Evaluation study of a cosmetic product for the treatment of wrinkles
(Questionario:Studio di valutazione soggettiva di un prodotto cosmetico nel trattamento delle rughe)

Età(Age): _____

Sesso(Sex): M ☐
 F ☐

Usa normalmente prodotti antirughe (Do you normally use an antiwrinkle product)?

 Si(yes) ☐
 no ☐

se si, quale(if yes, which one(s) ? _____

Efficacia cosmetica (cosmetic efficacy):
La prego di valutare le proprietà cosmetiche del prodotto in accordo con i seguenti punteggi
(Please evaluate the cosmetic properties of the product according to the following criteria):

1=molto efficace (very efficacy) 2=efficace (efficacy) 3=poco efficace(slightly effective)
4= per niente efficace(not at all effective) 5=senza opinione(no opinion)

Punteggio(Criteria)	1	2	3	4	5
Miglioramento dell'aspetto visivo della pelle (appearance of the skin)					
Effetto levigante (smoothing effect)					

 Come ha tollerato il prodotto(How did you tolerate the product)?

 Molto bene(very well) ☐
 Bene(well) ☐
 Abbastanza bene(fairly well) ☐
 Per niente(not at all) ☐
In caso di problemi quali reazioni ha avuto(in case of problems, which reactions)?

 _____

Gradevolezza cosmetica (cosmetic pleasantness):

 La prego di valutare le proprietà cosmetiche del prodotto in accordo con i seguenti punteggi
(Please evaluate the cosmetic properties of the product according to the following criteria):

1=molto buono(very good) 2=buono(good) 3=nè buono nè cattivo(neither good nor bad)
4=cattivo(bad) 5=molto cattivo(very bad) 6=senza opinione(no opinion)

DIPARTIMENTO DI CHIMICA FARMACEUTICA

UNIVERSITA' DEGLI STUDI DI PAVIA

Segreteria amministrativa

C.F.. 80007270186 P:.IVA 00462870189 Via Taramelli, 12 – 27100- Pavia – tel. 0382 987358 fax 0382 422975

Punteggio(Criteria)	1	2	3	4	5	6
Sensazione sulla pelle durante l'applicazione (skin feeling during application)						
Sensazione sulla pelle dopo l'applicazione(skin feeling after application)						
Profumo (parfum)						
Sensazione di idratazione sulla pelle dopo l'applicazione (Hydration feeling after application)						
Assorbimento sulla pelle (skin absorption)						
Spalmabilità (spreadability)						
Consistenza del prodotto (texture)						
Il prodotto in generale-commenti sul prodotto in generale(Product Overall (your mark for the product in general)						

E' soddisfatto delle proprietà cosmetiche(Are you satisfied with the cosmetic properties)?

Molto soddisfatto(very satisfied)	☐
Soddisfatto(satisfied)	☐
Non proprio soddisfatto(not really satisfied)	☐
Per niente soddisfatto(not satisfied at all)	☐

Comprerebbe il prodotto(Would you buy the product)?

Si(yes)	☐
no	☐
Senza opinione(no opinion)	☐

Altri commenti(Any additional comment)?