STUDY REFERENCE: 02/STG-1_399_19-001

Study Report - Version nº 1

EVALUATION OF THE STING RELIEF PRODUCED BY A COSMETIC PRODUCT

CLIENT: LABORATORIOS MONTPLET S.L.U.
TESTED PRODUCT: EMULSIÓN CALMANTE CON MENTOL
REFERENCE: ID058-CILMTI003

Madrid, September 19th 2019

REPORT INDEX

1. SYNOPSIS

SPONSOR	LABORATORIOS MONTPLET S.L.U. VIA TRAJANA 53-59
Tested product:	EMULSIÓN CALMANTE CON MENTOL
Date of order	August, 1st 2019
Testing Facility:	ZURKO RESEARCH S.L. Almansa, nº 110, local 18. 28040, Madrid (Spain). Tel: (+34) 91.521.15.88
Supervisors of Study	Ana García Blanco, Biologist
Study code	02/STG-1_399_19-001
Subjects	Number of Subjects enrolled: 28 Gender: both Age range: 18-70 years Skin type: sensitive skin according to center criteria Capsaicin sensitive Number of Subjects Completed: 21
Test area	Face (nasolabial folds)
Application	Duration: 3 days
Test period	September, 3rd 2019 - September, 12th 2019
Test parameters	Subjective evaluation of stinging feeling
Design of study	Day 01: selection of volunteers sensitive to capsaicin Day 03: evaluation of the stinging relief produced by a cosmetic product
Evaluation	Statistical analysis of stinging relief

Results:

After 2, 5, 10 and 15 minutes of product use, there was decrease in sting intensity with a reduction from control of 17%, 17%, 67% and 83% respectively. For each time compared to control area, the stinging relief is statistically significative relative to control area, with a p-value lower than 0.05.

2. IDENTIFICATION OF THE STUDY

Name of the study: Evaluation of the sting relief produced by a cosmetic product.

Director of the Laboratory: Irene Zaldívar Notario

Director of the study: María Barbero Calderón

Sponsor: LABORATORIOS MONTPLET S.L.U.

Sponsor's adress: VIA TRAJANA 53-59.

Tested product: EMULSIÓN CALMANTE CON MENTOL, reference: ID058-CILMTI003, batch: M16119.

3. OBJECTIVE AND PRINCIPLE OF THE STUDY

This study has as objective to evaluate the sting relief produced in 20 volunteers by a cosmetic product**.**

To assess the relief of stinging, a stinging test was performed by subjectively evaluating the intensity of stinging according to an ordinal scale with values ranging from 0 to 10, where: 0 being not feeling any stinging and 10 feeling a lot of stinging.

The evaluated product is emulsion.

4. TYPE OF STUDY

This test was performed under control by the center.

Each volunteer who has taken part in the study has been the own controller.

The study was carried out following general conditions in Zurko Research, established for the execution of study project on humans (Structure and Content of Clinical Study Reports from ICH Harmonised Tripartite Guideline; Guideline for good clinical practice E6 (R2) of June 14th 2017, EMA/CHMP/ICH/135/1995 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE – May 1st 2001).

Previously, Zurko Research assessed the suitability of the product for the type of study and methodology used.

5. RESEARCH CENTER

5.1. Research Center

ZURKO RESEARCH S.L.

Almansa Street, nº 110, local 18.

28040 Madrid (Spain)

Tel: (+34) 91.521.15.88

5.2. Researcher team

Study's director: María Barbero Calderón, Pharmacist.

Researcher: Ana García Blanco, Biologist.

Technician: Marina Gómez Izpurra.

Statistician: Raquel Chica Martínez.

6. STUDY'S SCHEDULE

Beginning of the experimental phase: September, 3rd 2019

End of the experimental phase: September, 12th 2019

*Due of different dates of the recruitment

7. VOLUNTEERS

7.1. Ethical aspects

Each volunteer participating in the study has been notified before about the type and the procedures of the study, signing an informed consent before the beginning of the study. The signed informed consents were archived in Zurko Research.

7.2. Number of volunteers

28 volunteers were included in the study. The number of volunteers required at the end of the study was 20, considering that the number of volunteers used in this type of study is sufficient to verify the relief of stinging by the product.

One volunteer (ref. V16) discontinued the study due to reasons unrelated to it. Six exclusions (V2, V14, V18, V19, V25 and V28) were recorded, the latter being decided by the principal investigator when the volunteer did not meet the inclusion criteria (sensitive to capsaicin).

Thus, relief of stinging has been studied in 21 volunteers.

Volunteers participating in the study met the inclusion criteria. Data on participating volunteers are included in Annex I.

7.3. Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were as follow:

- Gender: both.
- Age: 18-70 years.
- Sensitive skin according to criteria established by the center.
- Good health condition.
- Sensitive to capsaicin.
- Availability during the duration of the test.
- Well understanding of the information provided regards the objective and development of the study.
- Signature of the informed consent.

7.4. Specific exclusion criteria

- The absence of stinging when applying an aqueous solution of lactic acid, and/or presence of stinging when applying the saline solution.
- The presence of any pathology in the study area that may interfere with the study.
- To present relevant dermatological pathologies (Atopic dermatitis, Psoriasis, Lupus, Rosacea, Ringworm, etc.)
- Skin markings in the experimental area that may interfere in the evaluation of skin reactions (pigmentation, scars, excess hair in the area, excessive freckles and moles, sun burns…)
- Tattoos in the experimental area; injuries, pathologies or infections in the experimental area.
- Eczematous skin reactions which are not completely removed, and scars or skin-pigment complications in the experimental area from previous trials.

- Intensive sun or UV rays exposure on the experimental area either during the course of the trial or in the previous month.
- Volunteers who have been subjected to an extraction or transplantation of organs; volunteers who have suffered a cranial trauma with lengthy loss of consciousness in the last 5 years, or subjects with cranial trauma with current sequels.
- Volunteers who have:
    - Cardiovascular, digestive, neurological, psychiatric, genital, urinary, haematological or endocrine progressive alteration.
    - Immunodeficiency.
    - Previous history of medicinal, cosmetics, healthcare, household, industrial products (especially latex, aluminium or nickel) intolerance.
    - Previous history of allergies, photosensitivity or phototoxicity.
    - Progressive cutaneous alteration.
    - Progressive febrile process.
    - Metabolic photodermatitis: porphyria, tryptophan metabolism disorders.
- Exposure to antibiotics, antihistamines, corticosteroids, beta blockers, retinoid, azelaic acid, anti-acne treatments for which treatment is not completed during the 15 previous days to the study.
- Volunteers under any treatment for psoriasis or vitiligo during the previous month.
- Have been vaccinated within 3 weeks prior to the study.
- Being pregnant or breast-feeding.
- Apply other similar topical products in the experimental area during the study.
- Apply other cosmetics types not used usually in the experimental area.
- Present any type of allergy to the ingredients of the cosmetic product.
- Participation in any study that would interfere with the current study.
- Withhold the informed consent.
- Resistant skin.

The availability of the volunteers was reviewed and confirmed so as not to compromise the clinical and subjective evaluation at the end of the study.

8. METHODOLOGY

8.1. Criteria for the application of the product

Product type: leave on emulsion.

Experimental area: face.

Method of application: the treated area of the face was covered with the tested product.

Duration of the study: 3 days.

8.2. Experimental procedure

The participants of the test signed the informed consent.

For the sting relief, a subjective evaluation measure of the sting intensity was given according to an ordinal scale.

After 5 minutes from the application, the volunteers completed a questionnaire about the sting intensity they are experiencing.

Summary of the experimental procedure:

D1	28 volunteers signed the informed consent		
	Criteria verification	The researcher verified the specific inclusion and exclusion criteria of the volunteers.	
	Efficacy	**Sting detection**	Subjective evaluation of stinging intensity on an ordinal ten-point scale. 28 volunteers.
D3	**Efficacy**	**Sting relief**	Subjective evaluation of stinging intensity on an ordinal ten-point scale. 21 volunteers.

8.3. *Stinging test*: Evaluation of the effect on stinging after the use of a product

Environmental conditions

The days of measurement, the volunteers participating in the study were during 10-15 minutes in an acclimatized room, with these conditions: temperature 20ºC ± 2ºC and relative humidity 40-60%.

Subsequently, the volunteers were subjected to a facial sauna for 5 minutes.

Stinging intensity assessment

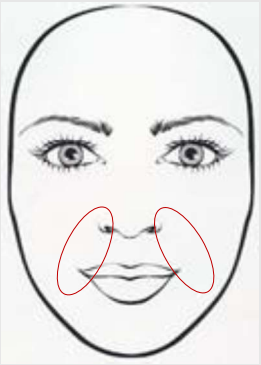
Basal Stinging: On the first day of the study (D1), the technician applied to volunteers:

- Enough quantity of cream with 0,075% capsaicin on the side of the treated face.
- Enough amount of base cream without capsaicin on the side of the control face.

After 5 minutes, the volunteers measured the stinging intensity on an ordinal rating scale with values ranging from 0 to 10, 0 being no sting and 10 the highest possible level of sting.

The investigator decided to exclude volunteers when either they did not notice enough stinging in the treated experimental area or stinging was reported in the control area.

Stinging after the product application: Next day (D3), enough quantity of cream with 0,075% capsaicin was applied to cover the experimental area of the selected volunteers. Once the stinging intensity reached the level of the first day (D1), enough quantity of the tested product was applied on the area. The volunteers indicated the intensity of the stinging in both areas at different times: one minute (T01), two minutes (T02), five minutes (T05), ten minutes (T10) and fifteen minutes (T15).

Stinging test		
Panel	Age Gender Number of volunteers	18 – 70 years Both 28
Experimental area	Anatomical location	Face, nasolabial furrows 
Environment conditions	Room temperature Ambient air relative humidity	20ºC 40–60%
Product	Amount of the product	Enough to cover the treated area
Experimental times	General Duration of the treatment before on application	Baseline time D1. D3: T0, T01, T02, T05, T10, T15.
Measurements	Number of consecutive measures	1
Statistical study	Analysis p-value	Cumulative logit mixed model (CLMM model) P<0.05 (confidence interval 95%)

8.4. Statistical study of the results

Descriptive statistical analysis was performed to the efficacy parameters at different experimental times, including the mean, median, standard deviation, maximum, minimum and variation between treated and control, and graphical representation. The intensity of stinging sense was measured according to an ordinal scale with values from 0 to 10, 0 being no stinging and 10 the highest possible level of stinging. Individual data are included in Annex II in absolute values for each experimental time.

The stinging grade is treated as an ordinal categorical response variable in a cumulative mixed model. Longitudinal data (same measurement obtained over time from the same volunteer), and thus correlated data, was taken into account by including random effects at the subject level and allowing varying intercepts across volunteers.

A significance level of 0.05 (95% confidence interval) was considered for the effect to be significant.

10. CONCLUSION

The purpose of this study was to determine the stinging relief efficacy of the **EMULSIÓN CALMANTE CON MENTOL** product, reference **ID058-CILMTI003**, through the statistical analysis of stinging intensity assessment in 20 volunteers.

Under the adopted experimental conditions and considering the defined instrumental parameters:

- **The cosmetic product had a significant calming effect after 2 minutes, with a tested reduction of 17% and with a 91% predicted probability of calming on treated area as compared to non-treated control area.**
- **The cosmetic product had a significant calming effect after 5 minutes, with a tested reduction of 17% and with a 94% predicted probability of calming on treated area as compared to non-treated control area.**
- **The cosmetic product had a significant calming effect after 10 minutes, with a tested reduction of 67% and with a 99% predicted probability of calming on treated area as compared to non-treated control area.**
- **The cosmetic product had a significant calming effect after 15 minutes, with a tested reduction of 83% and with a 100% predicted probability of calming on treated area as compared to non-treated control area.**

11. DOCUMENT CONSERVATION AND SAMPLES

The following documentation relating to the study will be stored in the facilities of Zurko Research following the provisions of ISO 9001:2015:

- Study protocol and its modifications (signed)
- Primary data
- Final Report
- Documents provided by the sponsor

The documentation will be stored for 5 years. At 5 years the possibility of an extension due to the commercialization of the test product will be consulted with the promoter.

A sample of the evaluated product (sufficient quantity for the execution of the study) will be stored in Zurko Research's library for 1 year from the date of receipt.

12. BIBLIOGRAPHIC REFERENCES

1. The SCCS´S Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation

2. www.cosmeticsinfo.org

3. Nueva Clasificación de tipos piel y sus implicaciones en Dermatología Cosmética. Revisión Dermatología Venezolana. Vol. 43, Nº 4, 2005. Leslie Baumann, Sadegh Amini, Eduardo Weiss.

SIGNATURES

Researcher: Ana García Blanco, Biologist. I, the undersigned, Ana García Blanco, declare that this study has been performed under my responsibility and in the essence of the Clinical Good Practices (Guideline for good clinical practice E6 (R2) of June 14th 2017, EMA/CHMP/ICH/135/1995 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE – May 1st 2001).

- The results here presented reflect accurately and completely the raw data of the study.

Signature:

Responsible of the Area of Quality Management: Andrea Gómez Herranz, **Quality Guarantee Technician.** I, the undersigned, Andrea Gómez Herranz, declare that this study has been carried out under my responsibility and under the principles of ICH Good Clinical Practice (Guideline for good clinical practice E6 (R2) of June 14th 2017, EMA/CHMP/ICH/135/1995 of May 1st 1996, European Parliament and Council Guideline 2001/20/CE – May 1st 2001).

- The inspections that have been made, allow confirming that the final report reflects accurately the primary data of the study.

Signature: