EVALUATION OF THE HYDRATION EFFICACY OF A COSMETIC PRODUCT AFTER APPLICATION UNDER DEFINED CONDITIONS IN NORMAL SUBJECTS

ACUTE HYDRATION - 48H

Objective evaluation of the hydration by "capacitance" method

Report (Version 1)	July 4th, 2013
Study Code	810613.B
Product	CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655
Sponsor	DMC - Donkey Milk Cosmetics, Lda Foros de Mora 7490-309 Mora
Study monitor	Dr. Jorge Leal BARRETO
Study Center	PhD Trials Campo Grande 286, R/C Dto e 4.º Dto. 1700-096 Lisboa
Scientific Manager **Principal Investigator**	Pedro Contreiras Pinto, *PhD* Leonor Girão, *MD*

The present study was performed according the criteria of Good Clinical Practices, and the Quality procedures implemented in PhD Trials.

This report has 33 pages

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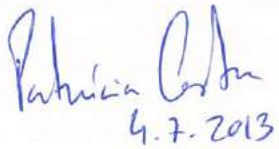
CERTIFICADO DE CONTROLO DE QUALIDADE

Quality Inspection Certificate

Study number	810613.B
Date of the beginning of the study	June 24th 2013
Date of the end of study	June 26th 2013
Date of audit	June 26th 2013

O estudo acima referenciado foi realizado de acordo com as regras das Boas Práticas Clínicas e com os procedimentos padronizados da PhD Trials.
The study above listed was performed according with the rules of Good Clinical Practices, and under the standardized procedures of PhD Trials.

O responsável pelo Sistema de Gestão da Qualidade atesta que o presente relatório está de acordo com os dados obtidos e que foi realizado de acordo com os procedimentos e regras acima referenciados.
The Quality System responsible certifies that this report is according with the obtained raw data and respects the procedures and rules above listed.

Name / Surname	Patrícia Costa, MsC *(Quality manager NP EN ISO 9001:2008; Quality internal auditor EN ISO 19011:2011)*
Date	July 4th, 2013
Signature	

STUDY SUMMARY REPORT

OBJECTIVE	The aim of the present study is to assess the **hydration efficacy,** after the application of the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655** in healthy human volunteers, at 24H and 48H after application.
STUDY DATES	**Beginning**: June 24th 2013 **End**: June 26th 2013
NAME **REFERENCE** **BATCH NUMBER**	CREME FACIAL LEITE DE BURRA - BATCH 330127/A 655
SUBJECT NUMBER	The test product was assessed in **fifty** (15) volunteers.
SPECIFIC INCLUSION CRITERIA	Age: 18 to 65 years, Gender: female, Phototype (Fitzpatrick) : I to IV, Type of skin: all types of skin
CONCLUSION	The product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 presented a statistically increase in the skin hydration after 24 hours of application of 22%, when in comparison against water, and a statistically increase in the skin hydration after 24 hours of application of 22.8%, when in comparison against the solvents, thus been considered hydrating. The product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 presented a statistically increase in the skin hydration after 48 hours of application of 29.5%, when in comparison against water, and a statistically increase in the skin hydration after 48 hours of application of 43.7%, when in comparison against the solvents, thus been considered hydrating. These results show that product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 strengthen skin hydration at 24H, after one application, and at 48H after two applications.

STUDY SCHEDULE

	Inclusion	T0	Start of counting time	T24H	T48H
Clinical history, informed consent	●				
Verification of inclusion/exclusion criteria	●				
Basal Hydration Measurement		●			
Application of test product and control		●		●	
Biometric measurement				●	●

I . OBJECTIVE

The aim of the present study is to assess the **hydration efficacy,** after the application of the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655** in healthy human volunteers, at 24H and 48H after application.

The experimental design considers the specific application conditions of the product. In this context, it adopts a widely used study model, which involves the application and measurement of skin hydration over time by "corneometry". This approach, allows for the quantitative description of such hydration, as well as a comparison against defined controls.

The hydration efficacy of the product is assessed:

- objectively and quantitatively, by instrumental measurements of the skin hydration taken in the forearm, with a Corneometer® (Cutometer® dual MPA 580), before and after treatment.

II . STUDY RELEVANCE

II. 1. Methodological approach

The experimental conditions adopted in this study involved the application of the product and its contact with the skin, for a certain period of time, thereby facilitating its penetration, and subsequent hydration.

The anatomical area chosen for the experimental testing (forearm) allows for the study product to be easily evaluated. The application was randomised in order to reduce the skin reaction variability dependent of the application zone.

All measurements involve a prior acclimatisation period and are performed in a controlled environment.

The proposed methodology results from the adaptation of several published works (see XII).

II.2. Panel

Volunteers were selected according to a number set by specific requirements (taking into account the experience acquired in other studies involving cosmetic products and knowledge of the constituents of the product formulas on study), as well as set criteria for inclusion and non-inclusion.

II.3. Results

Whenever appropriate, results are presented in absolute value and/or percentage change, in view of the study objectives.

The proposed descriptive and comparative statistics is obtained with MS Excel 2010 (Microsoft, USA) and SPSS 20th (IBM, USA).

II.4. Ethics

The study is performed according to the Declaration of Helsinki principles and subsequent amendments.

The object of the study consists in the application of the test product in accordance with its normal use, in order to reduce any possible risk to volunteers that may be selected for the trial. There is a commitment between the particular objectives of the study and any potential risks and problems related to the protocol on trial.

The application of the product was carried out by the responsible Investigator, at the Research Centre. The study was conducted in the spirit of Good Clinical Practice Guidelines and general principles of Law 46/2004 of August 19th. The protocol and test conditions were reviewed by the Internal Review Board (**opinion nº 470/13 from June 21st, 2013**) and the standard protocol was submitted to the Ethical Commission of PhD Trials (**opinion nº 1/2012 from January 26th, 2012**).

The risks incurred by volunteers in the development of this study were minor and without any clinical implications. The study may come to reveal previously acquired allergies to some of the ingredients. However even in such cases, the reaction should be limited to the test area, and its manifestations confined and controlled.

The research center was responsible for providing information and prior knowledge to all volunteers selected.

All the data concerned subject health and clinical data during and after the study performance are subject to medical-patient relationship. The investigational centre cannot send to the sponsor the actual identity of the subjects. The investigational centre will present the data fully codified in respect to subject's data.

III . TYPE OF STUDY

This project is a monocentric, open study.

The individual is used as its own control.

This study was conducted in accordance with the general conditions of PhD Trials, established for the development of a research project involving human subjects, summarised by protocol (MD.34/01).

IV . INVESTIGATION CENTER

IV.1. Study Center

PhD Trials: Clinical Center
Campo Grande, 286, R/C Dto e 4.º Dto
1700-096 Lisboa
Portugal

PhD Trials: Head-office
Rua do Centro, nº23
Casal de Sta Iria
2240-300 Chãos, FZZ
Portugal

Tel : + (351) 969104007
e-mail: geral@phdtrials.com

IV.2. Technical staff

Investigator (dermatologist): Leonor GIRÃO, *MD*
Scientific manager: Pedro CONTREIRAS PINTO, *PhD*
Quality manager: Patrícia COSTA, MsC
Administrative coordinator: António COSTA

V . DATES OF STUDY

Beginning: June 24th 2013

End: June 26th 2013

VI . PRODUCTS

VI.1. Identification of the test product

Name	**CREME FACIAL LEITE DE BURRA**
Reference	-
Batch number	A655
Galenic form and organoleptic characteristics	Pearl colour cream
Number and type of samples	2 samples
Volume of samples	50ml

The product was tested against a blank, non-treated site, a control with water and a control with solvents (50%/50% chloroform/alcohol).

VI.2. Information concerning the test product

The documents related with the test product, supplied with the samples, are: Letter of Agreement, particularly concerning the conformity of the formulae to the established regulations and their safety, and the Order Form. The qualitative and quantitative formula of the products can be requested by the Investigator in a case by case situation, especially if there are some reactions noted.

VII . SUBJECTS

VII.1 . Number

The number of volunteers whose data had to be exploitable at the end of the study was 10.

Fifty (15) volunteers were included in the study. There were no withdrawals and no exclusions.

The test product was therefore assessed in **fifty** (15) volunteers.

VII.2 . Specific inclusion criteria

The specific inclusion criteria, defined in the protocol, were the following ones:

a) Age: 18 to 65 years,

b) Gender: female,

c) Phototype (Fitzpatrick) : I to IV,

d) All types of skin

All the volunteers corresponded to these specific inclusion criteria. Their typological characteristics are defined in **Appendix 1.**

VII.3 . Specific non-inclusion criteria

The specific non-inclusion criteria are those defined for this kind of methodology in accordance with the corresponding procedure and the following ones for this study particularly:

a) cutaneous marks on the experimental areas, which could interfere with the assessment of skin reactions (pigmentation troubles, scar elements, over-developed pilosity, ephelides and naevi in too great quantity, sunburn),

b) allergy or reactivity to products of the same category than the tested one,

c) treatment with Vitamin A acid or its derivatives stopped less than 3 months before the beginning of the study,

d) forecast of intensive sun or UVA exposure (UV lamps) during the test period,

e) breast-feeding or pregnancy,

f) prolonged exposure to sun in the month preceding the study,

g) topical treatment using corticoids on the application zone in the eight days prior to starting the study,

h) UVA or UVB treatment in the month preceding the study,

i) application of hydrating products on the forearm

All the volunteers corresponded to these specific non-inclusion criteria.

VIII. METHODOLOGY

VIII.1 . Experimental area and sites of application of the test products

The chosen area of experiment was the forearm.

The area of application and control were randomly selected by the researcher.

The test product was applied by the researcher to one of the sites localized by a clockwise distribution, altering of one rank from a subject to another.

VIII.2 . Experimental conditions of application of the test products

Product name	Product directions of use	Quantity to be applied
CREME FACIAL LEITE DE BURRA	In the study centre, by the investigator, in an area of $1cm^2$	10 mg/cm^2

The amount of applied product was measured using a disposable syringe.

In order to ensure uniformity of test procedures on all volunteers, application of the test product was carried out by the researcher.

VIII.3 . Chronology of the study

The constraints imposed on the subjects during the test product are the following ones:

a) no application of products on the experimental area (except the tested products),

b) full respect of the test product conditions of use,

c) no application of hydrating products on the experimental areas,

d) no change in hygiene habits,

e) tight clothing should not be used around the study zone, in order to avoid friction,

f) during the testing period, one should refrain from being too exposed to water (i.e.: immersion bath, bathing in the sea or pool), sauna or Turkish baths;

g) avoid prolonged exposure to sun or UVA rays (UV lamps) during the study period in particular the day before applying the products.

h) neither anti-allergic, anti-inflammatory treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen),

VIII.4 . Control of the observance of the modalities of the protocol

The investigator checked about the respect of the **constraints**.

The volunteers were questioned at the end of the study. The Investigator assessed the importance of the possible deviations in comparison with the experimental conditions required at the beginning of the study.

The synthesis of the answers obtained was enclosed in **Appendix 2**.

All the deviations from the protocol were analyzed and the investigator assessed their effect on the validity of the results.

All the constraints of the study, defined in the protocol, were respected by the volunteers.

VIII.5 . Checking of the acceptability

VIII.5.1 . Frequency of the examinations

A skin examination of the experimental areas was performed by the dermatologist or the responsible technician.

This examination was performed visually under standard "daylight" source, before then after 24H and 48H of the product application.

Concurrently with the clinical examination(s) performed, each subject was questioned about the possible sensations of discomfort he felt.

VIII.5.2 . Expression of the results of the skin examination and questioning

The information gathered during the questioning was compared to that referenced by the subject.

The expression of the results of the skin examination and questioning was that defined for this type of study in accordance with the corresponding procedure.

The subject should referred any reaction or sensation of discomfort on the sheet reserved for that effect, using his own words to express what he feels.

In case of reactivity:

- the main sensations of discomfort are described, i.e. :

Heating, Stinging, Pruritus (itching), Pulling and Burning.

The intensity of the sensations of discomfort is assessed according to an ordinal scale: slight, moderate, severe.

- the main visible signs are noted, i.e. :

Erythema, Oedema, Vesicle, Bulla, Papule, Scab, Dryness, Coloration and Macula.

The intensity of the erythema and oedema is assessed according to an ordinal scale: slight, moderate, severe.

The appearance of the erythema is specified: diffuse, punctuated.

The importance of the number of vesicles is assessed according to an ordinal scale: 1 to 2 vesicles, more than 2 vesicles.

The papules, bullae and maculae are counted.

The scabs, dryness and coloration are described.

The importance of the dryness and coloration is assessed according to an ordinal scale: slight, moderate, severe.

The dermatologist or the responsible technician notes for any clinical sign or sensation of discomfort describing its location, duration, date of occurrence after application of the product, frequency, intensity, evolution, medical treatment possibly undertaken, relation to the product : none, doubtful, possible, obvious.

He also notes the usual or unusual characteristic of the clinical sign or sensation of discomfort described, by questioning the subjects about the effects observed when applying similar products.

The results are expressed in a descriptive manner for the visible signs or the sensations of discomfort and the percentage of "reactive" subjects is calculated.

VIII.5.3 . Interpretation of the results of the skin examination and questioning

All the subjects included in the study are taken into account to check the acceptability of the test product as long as they are submitted at least to one post application examination at the defined time.

The interpretation of the results of the questioning and skin examination and of the data gathered in the observation sheet is that defined for this type of study in accordance with the corresponding procedure.

This interpretation, performed by the dermatologist is absolute, referring to the following grading scale, which takes into account the sensations of discomfort described by the subjects and the visible reactions of irritation (clinical signs). The test product can therefore have a very good, good, moderate or bad acceptability.

Acceptability	% of subjects exhibiting clinical signs ascribable to the test product	% of subjects exhibiting sensations of discomfort ascribable to the test product
Very good	0 %	0 %
Good	0 %	< 25 %
Moderate	< 10 %	Any
	0 %	25 to 50 %
Bad	≥ 10%	Any
	0 %	> 50 %

According to the nature and intensity of the clinical signs or sensations of discomfort, the dermatologist can have to under-class or over-class the product in relation to the grading scale.

If an allergy to the test product is suspected, an additional study can be required and performed after agreement by the Sponsor; for example, allergologic inquiry (patch test) to search for the responsible ingredient(s) or confirmation of the reactivity of the skin to the product concerned (open test). The modalities of the additional study are defined by the dermatologist.

The interpretation of the results is also relative referring to the internal data base of the investigator centre.

VIII.6 . Assessment of the efficacy

VIII.6.1 . General conditions of the study

VIII.6.1.1 Standardization of the room conditions

All the evaluations were performed in a fully controlled room and after an initial acclimatization process of at least 20 min in a fully controlled and acclimatized room (Controlled temperature: T = 22°C ± 2°C; Controlled relative humidity: RH = 50% ± 5%).

A summary of the room temperature and relative humidity during the study was presented in **Appendix 3.**

VIII.6.1.2 Study schedule

The sequence of evaluations was as following:

	Inclusion	T0	Start of counting time	T24H	T48H
Clinical history, informed consent	●				
Verification of inclusion/exclusion criteria	●				
Basal Hydration Measurement		●			
Application of test product and control		●		●	
Biometric measurement				●	●

Application area: 1cm^2 square area in the forearm (defined by a standard mold)

Quantity of product to be applied: 10 mg/cm^2 of each product or control.

VIII.6.2 . Assessment of the skin hydration

VIII.6.2.1 Principle

Hydration content is obtained by an electrometric system which is based in the "Capacitance" measurement, allowing the calculation of the water dielectric constant. The measuring probe has an interdigital grid of gold-covered electrodes. The interdigital electrode is covered by a low dielectric vitrified material. Therefore there is no galvanic contact between the electrode and skin surface. A constant pressure is applied on the skin surface through a spring system. As a consequence of the probe design, the system (electrode, superficial parts of the stratum corneum and epidermis) behaves as a capacitor. Measurements are obtained in Arbitrary Units (AU) as reference to a Factory standard.

VIII.6.2.2 Equipment

The measurements were performed with a Corneometer CM825 probe, connected to a Cutometer® dual MPA 580 (Courage & Khazaka, Germany). Each defined area was evaluated (including the control).

VIII.6.2.3 Frequency of measurements

The measurements were performed at T0, T24H and T48H for each evaluation area obtained in sequence. The Basal of each one of the sub-areas were obtained at T0 and a mean was calculated to be used as the single Basal value in all the analysis, including the statistical calculations.

VIII.6.2.4 Expression and interpretation of the results

Each feature and area inside the defined area was calculated in each evaluation period.

The individual results are expressed:

a) in absolute values of the parameter for each experimental time,

b) in variation of the parameter against T0 for each experimental time.

c) in % change of the parameter values and area variation of T24H and T28H.

d) in comparison against the blank control.

The homogeneity of the forearm areas was confirmed by a statistical test performed at T0.

VIII.7. Statistical analysis

Statistical analysis was specifically applied to the numeric data resulting from the biometric evaluation, focusing on the absolute results of each data during the study.

At any stage of the study, data was gathered on all skin areas exposed to the test product and respective controls.

Descriptive statistics included the calculation of mean values, standard deviation, median and percentage related to the changes occurred following application of the product (reference to T0).

The % change regarding each control was calculated according with the following formulas:

$$\Delta = (ZTti - ZTt0) - (ZNTti - ZNTto) \text{ (em UA)}$$

$$\Delta\% = \left(\frac{(ZTti - ZTt0) - (ZNTti - ZNTto)}{ZTt0 + (ZNTti - ZNTt0}\right) x100$$

ZT: Treated area
t0: Basal
ZNT: Non treated area
ti: time of each evaluation.

A normality evaluation of the raw data and of the differences against the control was performed in order to define the best statistical procedure to perform the product comparisons.

A comparative analysis performed by the Wilkoxon Signed Ranks Test for paired data. A significance level adopted was 95%. The levels of hydration were compared after 24H and 48H with the Basal and between the test product and the controls.

IX . RESULTS

The results related with skin acceptability are presented in the **Appendix 4**. No skin reaction was noted after the application of the product. No volunteer experienced any discomfort during the study. Therefore the product presented **very good** skin compatibility during the study.

Skin hydration results of each individual volunteer are presented in **Appendices 5.1 to 5.4.** Figure 1 shows the summary of the absolute results evaluation for the product and the controls for each time points.

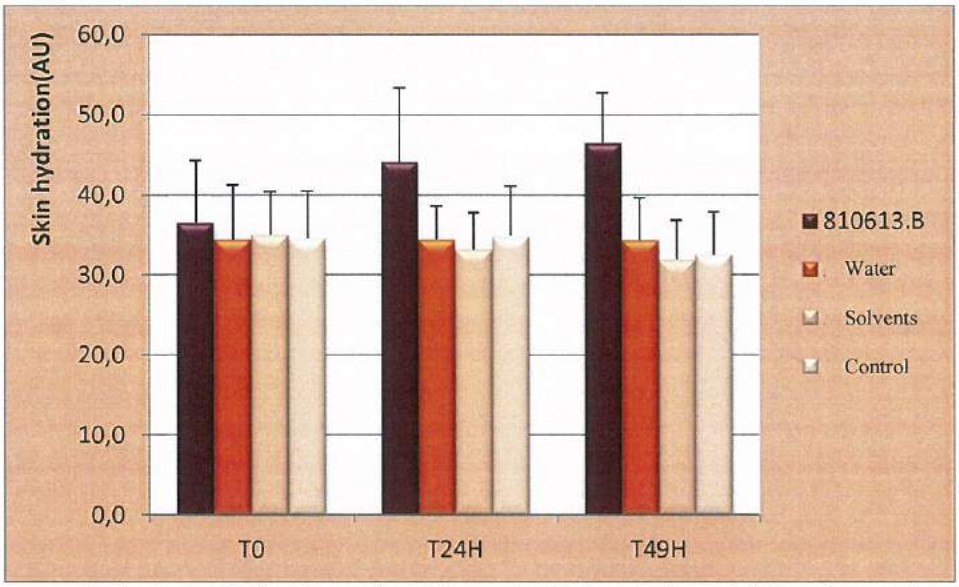


Figure 1 - Skin hydration (AU) evolution during the study. Mean + sd values of all the volunteers (n=12). Also shown is the statistical comparison against the control (*: p<0,05; N.S.: Non-significant)

In summary, at 24H and 48H, as a result of the application, there is a statistically significant increase of the hydration values in product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655**. As expected the solvents area reduces its value as time passes by. The non-treated zone maintains its value, close to the initial one (control).

After 24H from the application, the hydration did increased (p=0,001) and is also statistically different form the basal values, in the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655,** probably due to the presence of Donkey Milk. After 48H, the hydration increases (p=0,001), as result of a second application of the product. This simulated the normal use of the product at home, by the subjects, with one daily application. The controls did maintain their value as expected: the solvents with low values of hydration and the blank and water zones with values similar to the initial ones, guaranteeing that no environmental changes did affect the study.

A homogeneity test at time point 0 (Basal) was performed in order to guarantee that all the sites are statistically equivalent. A non-significant result was obtained (p=0,417), confirming that any changes are only related to the product application.

To infer about the capacity of hydration product at 24H and 48H, the absolute results were changed into variation percentages relative to controls "Water" and "solvents" results, according to the formulas of VIII.8. The results are shown in **Appendix 6** and summarized in the following charts:

Regarding control "Water":

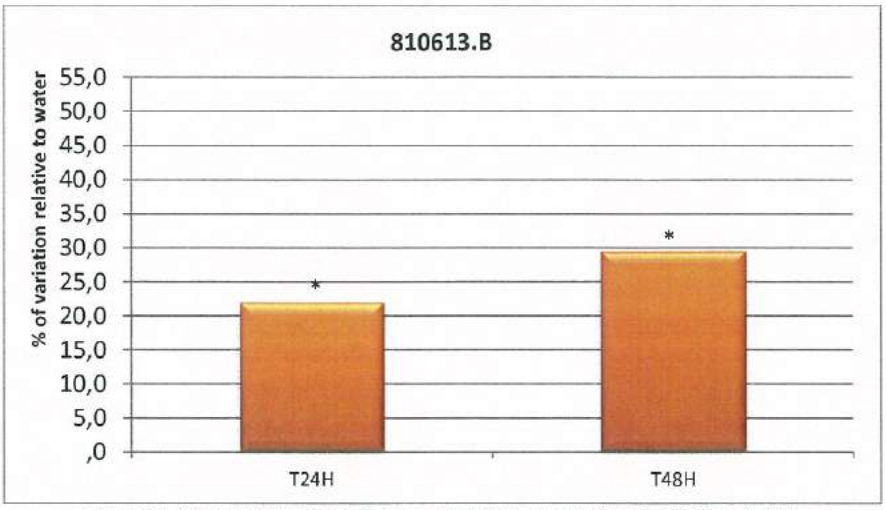


Figure 2 - % variation toward the control Water, obtained at 24H and 48H.
Mean values of all the volunteers. Also shown is the statistical comparison concerning Water, obtained with the absolute values (*: p<0,05; N.S.: Non-significant)

Table 1 - % variation toward the control Water, obtained at 24H and 48H.
Mean values of all the volunteers.

Product	T24H	T48H
810613.B	22,0	29,5

When in comparison with the simple application of Water, the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655** presented a statistically increase in the skin hydration, after 24 hours of application of 22%. This value increases to 29.5% at 48H. In this case, it is possible to state that the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655** presents an effective reinforce of the hydration after 48H.

Regarding to "Solvents":

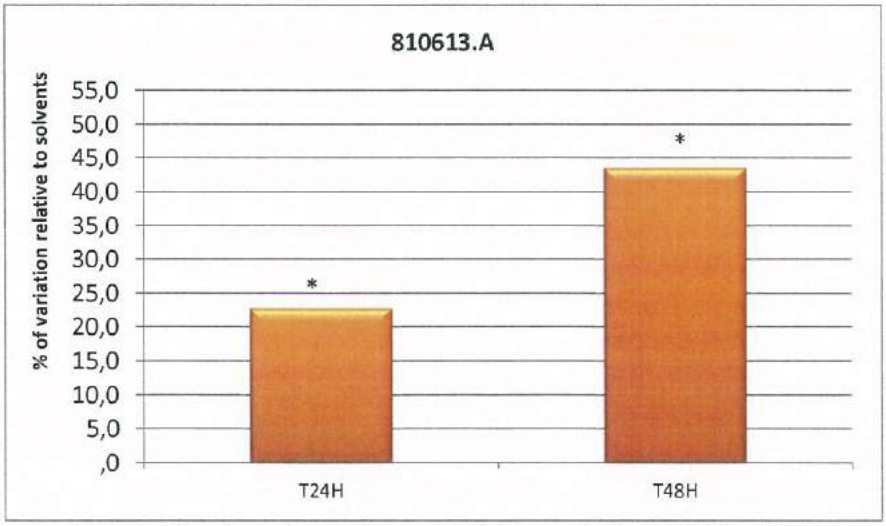


Figure 3 - % variation toward the control Solvents, obtained at 24H and 48H.
Mean values of all the volunteers. Also shown is the statistical comparison concerning Solvents, obtained with the absolute values (*: p<0,05; N.S.: Non-significant)

Tabela 2 - % variation toward the control Solvents, obtained at 24H and 48H.
Mean values of all the volunteers

Product	T24H	T48H
810613.B	22,8	43,7

When in comparison against the solvents, the product **CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655** presented a statistically increase in the skin hydration, after 24 hours of application of 22.8%. This value increases to 43.7% at 48H. Comparison with solvents allows to evaluate the performance of products in situations of extreme dehydration or dry skin and the ease that these products have on rehydrate the stratum corneum. In this case, it is found that the test product was effective in this context.

X . CONCLUSION

Under the experimental standardized conditions adopted and after all the statistical analysis we concluded that:

The product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 presented a statistically increase in the skin hydration after 24 hours of application of 22%, when in comparison against water, and a statistically increase in the skin hydration after 24 hours of application of 22.8%, when in comparison against the solvents, thus been considered hydrating.

The product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 presented a statistically increase in the skin hydration after 48 hours of application of 29.5%, when in comparison against water, and a statistically increase in the skin hydration after 48 hours of application of 43.7%, when in comparison against the solvents, thus been considered hydrating.

These results show that product CREME FACIAL LEITE DE BURRA, BATCH 330127/A 655 strengthen skin hydration at 24H, after one application, and at 48H after two applications.

XI . DATE AND SIGNATURES

4/7/2013

Investigator / Scientific Manager : Pedro CONTREIRAS PINTO

I the undersigned, Pedro CONTREIRAS PINTO, declare that the overall conduct of the study was carried out under my responsibility having in mind the basic principles and spirit of Good Clinical Practices ("Avis aux promoteurs et aux investigateurs pour les essais cliniques des médicaments" : principes généraux – FR.OB – 1987, international recommendations ICH E 6, step 4, of 1/5/1996 and general principals of the Portuguese law 46/2004 from August 19th).

PharDevelopment Trials, Lda
NIF: 509753914
Rua do Centro, 23 2240-300 Chãos

XII . REFERENCES

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APPENDICES

Appendix 1

TYPOLOGICAL CHARACTERISTICS OF THE SUBJECTS

Volunteers		Age (years)	Sex F=female M=male	Phototype[1]
Ref.	Codification[2]			
1	CABA. CE	23	M	III
2	CRUZ. CL	20	F	II
3	MESQ. FI	26	F	III
4	BAPT. CL	23	F	III
5	NUNE. EL	23	F	III
6	AMAD. PA	25	F	III
7	LOPE. MA	33	F	IV
8	SANT. BR	20	F	III
9	LOPE. RU	19	F	III
10	DORE. FA	18	M	III
11	FERN. RI	21	M	III
12	RAMO. AD	18	M	III
13	MONT. VI	20	M	IV
14	DESS. PU	31	F	IV
15	RAMA. AN	18	F	III

[1] ***phototype according to Fitzpatrick***, *established on the principle of a first 30 to 40-minute sun exposure after the winter or a period without exposure of an equivalent duration:*

Type I	*: Always burns easily, never tans*
Type II	*: Always burns easily, tans minimally*
Type III	*: Burns moderately, tans gradually*
Type IV	*: Burns slightly, always tans easily*
Type V	*: Burns rarely, tans intensely*
Type VI	*: Never burns, strongly pigmented*

[2] Volunteer code formation: 4 letters of surname + 2 letters of the name

Appendix 2

CONTROL OF THE OBSERVANCE - Constraints

Constraints (15 exploitable results)	Number of volunteers who respected the constraints	Percentage of volunteers who respected the constraints
No application of other products than the tested ones to the experimental area Deviation : none	15	100%
Full respect of the test product conditions of use Deviation : none	15	100%
No application of hydrating products on the experimental areas Deviation : none	15	100%
No change in hygiene habits Deviation : none	15	100%
Tight clothing should not be used around the study zone, in order to avoid friction Deviation : none	15	100%
During the testing period, one should refrain from being too exposed to water (i.e.: immersion bath, bathing in the sea or pool), sauna or Turkish baths Deviation : none	15	100%
Avoid prolonged exposure to sun or UVA rays (UV lamps) during the study period in particular the day before applying the products Deviation : none	15	100%
Neither anti-allergic, anti-inflammatory treatment nor treatment with patent medicines containing Vitamin A acid or its derivatives during the study (if therapeutic requirement : exclusion foreseen) Deviation : none	15	100%

Appendix 3

ROOM TEMPERATURE CONDITIONS

DATE	TIME	TEMPERATURE (ºC)	RELATIVE HUMIDITY (%)
24/06/2013	09:10:28	21,6	55,0
24/06/2013	17:10:28	21,6	55,0
25/06/2013	09:40:28	22,5	51,4
25/06/2013	17:10:28	23,2	51,7
26/06/2013	09:10:28	23,2	48,6
26/06/2013	17:10:28	23,0	53,7

Appendix 4

CUTANEOUS EVALUATION AND QUESTIONNAIRE DURING THE STUDY

Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	/	/	/
2	CRUZ. CL	/	/	/
3	MESQ. FI	/	/	/
4	BAPT. CL	/	/	/
5	NUNE. EL	/	/	/
6	AMAD. PA	/	/	/
7	LOPE. MA	/	/	/
8	SANT. BR	/	/	/
9	LOPE. RU	/	/	/
10	DORE. FA	/	/	/
11	FERN. RI	/	/	/
12	RAMO. AD	/	/	/
13	MONT. VI	/	/	/
14	DESS. PU	/	/	/
15	RAMA. AN	/	/	/

Appendix 5/1

RAW DATA – Product 810613.B

Subjects		Hydration (UA)		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	39,7	56,0	62,8
2	CRUZ. CL	33,1	34,1	38,1
3	MESQ. FI	28,1	34,4	35,9
4	BAPT. CL	32,2	39,2	47,4
5	NUNE. EL	44,1	45,3	58,9
6	AMAD. PA	35,2	41,8	48,5
7	LOPE. MA	40,7	42,9	45,6
8	SANT. BR	38,3	51,0	52,2
9	LOPE. RU	26,2	47,3	51,9
10	DORE. FA	36,3	38,6	43,8
11	FERN. RI	21,6	30,9	42,3
12	RAMO. AD	23,6	26,5	39,1
13	MONT. VI	29,6	65,1	55,9
14	DESS. PU	29,8	30,1	35,6
15	RAMA. AN	37,1	45,5	40,6
Mean		33,0	41,9	46,6
SD		6,5	10,4	8,4

Appendix 5/2

RAW DATA – Water

Subjects		Hydration (UA)		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	35,5	40,1	39,7
2	CRUZ. CL	28,6	28,9	28,3
3	MESQ. FI	29,4	30,7	29,5
4	BAPT. CL	30,8	33,5	31,4
5	NUNE. EL	44,4	46,3	47,8
6	AMAD. PA	34,6	35,1	39,3
7	LOPE. MA	40,7	36,4	36,6
8	SANT. BR	39,9	32,5	31,9
9	LOPE. RU	33,7	35,8	37,0
10	DORE. FA	39,3	32,6	29,2
11	FERN. RI	31,0	34,7	35,0
12	RAMO. AD	25,6	32,6	32,3
13	MONT. VI	27,3	32,6	33,9
14	DESS. PU	26,5	32,1	35,3
15	RAMA. AN	48,2	32,4	27,0
Mean		34,4	34,4	34,3
SD		6,9	4,2	5,4

Appendix 5/3

RAW DATA – Solvents

Subjects		Hydration (UA)		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	35,4	35,1	35,4
2	CRUZ. CL	31,8	28,1	28,2
3	MESQ. FI	29,0	27,4	26,8
4	BAPT. CL	31,8	31,0	31,9
5	NUNE. EL	44,5	39,8	38,8
6	AMAD. PA	36,2	35,9	35,2
7	LOPE. MA	44,0	40,9	34,4
8	SANT. BR	43,3	39,8	33,1
9	LOPE. RU	31,6	31,1	32,3
10	DORE. FA	36,8	32,1	18,4
11	FERN. RI	37,8	37,4	37,2
12	RAMO. AD	29,3	29,9	33,2
13	MONT. VI	28,8	27,7	32,9
14	DESS. PU	29,9	31,7	31,9
15	RAMA. AN	34,7	28,4	28,0
Mean		35,0	33,1	31,8
SD		5,5	4,7	5,0

Appendix 5/3

RAW DATA – Control

Subjects		Hydration (UA)		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	34,8	36,7	36,5
2	CRUZ. CL	26,6	18,3	18,9
3	MESQ. FI	26,2	26,8	24,9
4	BAPT. CL	30,7	32,0	33,3
5	NUNE. EL	44,4	41,3	41,1
6	AMAD. PA	36,1	37,2	38,3
7	LOPE. MA	42,9	37,2	32,2
8	SANT. BR	42,3	39,2	31,8
9	LOPE. RU	38,4	37,8	37,7
10	DORE. FA	38,2	36,4	29,8
11	FERN. RI	27,9	30,6	32,6
12	RAMO. AD	29,7	37,1	31,4
13	MONT. VI	31,1	31,5	32,9
14	DESS. PU	33,3	35,8	34,2
15	RAMA. AN	36,3	44,1	31,6
Mean		34,6	34,8	32,5
SD		5,9	6,3	5,4

Appendix 6/1

RELATIVE DATA – Product 810613.B

Subjects		% variation relative to Water		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	0,0	26,4	43,1
2	CRUZ. CL	0,0	1,9	16,2
3	MESQ. FI	0,0	17,0	27,3
4	BAPT. CL	0,0	12,3	44,5
5	NUNE. EL	0,0	-1,5	24,0
6	AMAD. PA	0,0	17,1	21,6
7	LOPE. MA	0,0	17,8	24,6
8	SANT. BR	0,0	64,9	72,1
9	LOPE. RU	0,0	66,8	75,6
10	DORE. FA	0,0	21,9	34,4
11	FERN. RI	0,0	22,1	65,0
12	RAMO. AD	0,0	-13,4	29,2
13	MONT. VI	0,0	86,7	54,5
14	DESS. PU	0,0	-15,0	-7,8
15	RAMA. AN	0,0	113,5	154,7
Mean		0,0	29,2	45,3
SD		0,0	37,2	37,7

Appendix 6/2

RELATIVE DATA – Product 810613.B

Subjects		% variation relative to Solvents		
Ref.	Name Surname	T0	T24H	T48H
1	CABA. CE	0,0	42,1	58,2
2	CRUZ. CL	0,0	-18,1	29,2
3	MESQ. FI	0,0	29,8	38,6
4	BAPT. CL	0,0	21,0	46,7
5	NUNE. EL	0,0	15,0	53,4
6	AMAD. PA	0,0	61,9	73,1
7	LOPE. MA	0,0	2,7	83,3
8	SANT. BR	0,0	27,9	75,6
9	LOPE. RU	0,0	-2,7	43,2
10	DORE. FA	0,0	109,5	124,0
11	FERN. RI	0,0	-20,9	18,4
12	RAMO. AD	0,0	2,2	36,8
13	MONT. VI	0,0	113,4	49,5
14	DESS. PU	0,0	-14,1	12,2
15	RAMA. AN	0,0	1,4	33,2
Mean		0,0	24,7	51,7
SD		0,0	41,9	28,4

Appendix 7

STATISTICAL ANALYSIS

Statistical results supporting figures 2 and 3 and text.

Statistical test for basal value homogeneity

Test Statistics[a]

N	15
Chi-Square	3,304
df	3
Asymp. Sig.	,347

Comparisons between each time and its Basal (absolute values)

Test product

Test Statistics[a]

	Prod860613t24H - Prod810613t0	Prod860613t48H - Prod810613t0
Z	-3,408[b]	-3,408[b]
Asymp. Sig. (2-tailed)	,001	,001

Controls

Water

Test Statistics[a]

	aguat24H - aguat0	aguat48H - aguat0
Z	-,625[b]	-,568[b]
Asymp. Sig. (2-tailed)	,532	,570

Solvents

Test Statistics[a]

	solvt24H - solvt0	solvt48H - solvt0
Z	-2,613[b]	-1,664[b]
Asymp. Sig. (2-tailed)	,009	,096

White

Test Statistics[a]

	brancot24H - brancot0	brancot48H - brancot0
Z	-,199[b]	-,966[c]
Asymp. Sig. (2-tailed)	,842	,334

Comparisons between each product to the control – Water and solvents

Test Statistics[a]

	aguat24H - Prod860613t24H	aguat48H - Prod860613t48H
Z	-2,556[b]	-3,408[b]
Asymp. Sig. (2-tailed)	,011	,001

Test Statistics[a]

	solvt24H - Prod860613t24H	solvt48H - Prod860613t48H
Z	-2,783[b]	-3,408[b]
Asymp. Sig. (2-tailed)	,005	,001

Appendix 8

ETHICAL COMISSION PROTOCOL OPINION

Comissão de Ética Independente

Independent Ethics Committee

Opinion nº 001/2012

A Comissão de Ética reviu o seguinte projecto / *The Ethics Committee reviewed the following project*:

Tipo de estudo / *Type of Study*	**Eficácia Hidratante Aguda/Acute Hydration Efficacy**
Nome do protocol / *Protocol name*	**ESTUDO *IN VIVO* PARA A DEMONSTRAÇÃO DA EFICÁCIA HIDRATANTE DE PRODUTOS COSMÉTICOS EM VOLUNTÁRIOS SAUDÁVEIS – HIDRATAÇÃO AGUDA / *In vivo* study for hydration efficacy claim substantiation in cosmetic products in healthy voluntaries – acute hydration**
Código do protocol / *Protocol Code*	IMP 01.02.03 vers.01

Documentos revistos / *Reviewed Documents*	☒ **Protocolo do estudo / Study protocol**
	☒ **Declaração de Consentimento Informado / Informed Consent Form**
	☒ **Formulário de Submissão / Submission form**

Decisão / *Decision*:
Após o cumprimento dos requisitos processuais e outras condições impostas por esta comissão, o projecto é: / *Subsequent to the fulfillment of the procedural requirements and other conditions imposed by the committee the project is hereby:*

Aceite / *Accepted*	☒	**Motivo da rejeição / *Motif to reject***
Aceite com modificações / *Accepted with modifications*	☐	
Rejeitado / *Rejected*	☐	

O investigador principal e os promotores deverão ler e cumprir com o seguinte: / The principal investigator and the sponsors are required to read and comply with the following:

- A Comissão examinou os procedimentos para o trabalho aqui proposto. Estes não apresentam impedimentos éticos à sua realização pelo os aprova neste estrito sentido. Todos os outros requisitos

relacionados com o trabalho (p.ex. científicos, processuais, legais, financeiros e regulamentares, etc) mantêm-se da única responsabilidade do investigador principal e/ou do promotor(es).

- *The Committee examined the proceedings for the work proposed concerning the ethical aspects and did not found any impeachment for their execution, therefore, approved them in this strict sense. All other concerns related to the work (e.g. scientific, procedural, legal, financial and regulatory etc) remain the sole responsibility of the principal investigator and/or the sponsor(s).*

Assinatura e data **Date and Signature**	26/01/2012
Responsible for the review	**Amilcar ROBERTO, PhD** Presidente da Comissão de Ética Chairman of the Ethical Committee