

PENTAPHARM

VIALOX[®] POWDER

Substantiation File

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Substantiation File

The following study reports are parts of this document and demonstrate the efficacy of VIALOX[®] POWDER:

1. Biological activity of pentapeptide 099-12 (VIALOX[®] POWDER) on the contraction frequency of muscle cocultured with spinal cord explant

The aim of this study was to analyze, in a coculture of muscle cells and spinal cord explants, the frequency contraction of muscle cells after 1 minute and 2 hours of incubation.

In conclusion, pentapeptide 099-12 significantly reduces muscle cell contraction.

2. Evaluation, on volunteers, of the anti-wrinkle effect of VIALOX[®] POWDER “Botox-Like” cosmetic treatment

The aim of the study was to evidence, on volunteers, the anti-wrinkle effect of VIALOX[®] POWDER after 28 days of twice-daily use using Primos[®].

Results showed up to -49% of wrinkle size and -47% of skin roughness after 28 days application.

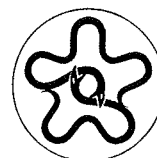
Conclusion

VIALOX[®] POWDER– the anti-wrinkle breakthrough without painful injections:

- VIALOX[®] POWDER peptide significantly reduces muscle cell contraction.
- Up to 49% decrease in wrinkle size measured with the VIALOX[®] POWDER kit.

5612 - bwa

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1. Biological activity of pentapeptide 099-12
(VIALOX[®] POWDER) on the contraction
frequency of muscle cocultured with spinal
cord explant

Proposal n°: RS040103

Study n°: RS040103

**BIOLOGICAL ACTIVITY OF PENTAPEPTIDE 099-12 ON
THE CONTRACTION FREQUENCY OF MUSCLE
COCULTURED WITH SPINAL CORD EXPLANT**
Botox-like effect

Final report of the study RS040103
Second version

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date: April 2nd, 2004

The investigators and the author of this report hereby certify the validity of the data presented and attest their full agreement with the conclusions presented at the end of the report.

Certified by:

Name: R. STEINSCHNEIDER

Position: Manager, pharmaceutical division

Date: April 2nd, 2004

Signature

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1 - INTRODUCTION

PENTAPHARM has the compound “**pentapeptide 099-12**” that could have Botox-like effect.

The aim of this study was to analyze, in a coculture of muscle cells and spinal cord explants, the frequency contraction of muscle cells after 1 minute and 2 hours of incubation.

In a first culture of human muscle cells, the cytotoxicity of the products was evaluated in order to determine the nontoxic maximum concentration to use for the continuation of the tests.

2 - MATERIALS AND METHODS

2.1. Biological model

2.1.1 Cytotoxicity

- Type: Normal human muscle cells (myoblastes : M1a, 3rd passage).
- Culture medium: Mix of 2/3 MEM (Invitrogen 21090-022) and 1/3 M199 (Invitrogen 31153-026)
L-glutamine 2mM (Invitrogen 25030024)
Penicillin 50 UI/ml - Streptomycin 50 µg/ml (Invitrogen 15070063)
Fetal calf serum 5 % (v/v, Invitrogen 10270098)
- Culture : 37° C and 5% CO₂

2.1.2. Assay

- Type: Normal human muscle cells (myoblastes : M1a, 3rd passage).
Explants of 13-day-old rat embryo spinal cord with dorsal root ganglia attached
- Culture medium: Mix of 2/3 MEM (Invitrogen 21090-022) and 1/3 M199 (Invitrogen 31153-026)
L-glutamine 2mM (Invitrogen 25030024)
Penicillin 50 UI/ml - Streptomycin 50 µg/ml (Invitrogen 15070063)
Fetal calf serum 5 % (v/v, Invitrogen 10270098)
- Culture : 37° C and 5% CO₂

2.2. Test compounds, reference

Test compounds	Stock solution	Dilution	Final concentrations tested
Pentapeptide 099-12 (RS040103/D, PM 615.7)	104.3 mg of compound dissolve in 6.8 ml of mix MEM/M199 (25mM)	In culture medium	5 mM

Reference	Stock-solution	Dilution	Final concentration tested
Carisoprodol (Sigma C-8759)	1 M in ethanol	In culture medium	0.1 mM and 0.01 mM

2.3. Cytotoxicity

Preliminary cytotoxicity assay was performed by the MTT assay for each compound to determine the non-cytotoxic limit of the compound to be assayed.

- plates: 96-well plates
- pre-culture: 72 h
- cells/well: 4 700
- tested concentrations: 8 (see table 1)
- replicates: 6
- cells/compound contact: 48 h
- Evaluation parameter: MTT hydrolysis assay

2.4. Human muscle cells and rat spinal cord explants coculture

Human muscle cultures were cultured in gelatin-coated 24-well plates. After muscle cell fusion showing myo-fibers without contractile activity, one 13-day-old rat embryo spinal cord explant with dorsal root ganglia attached was placed on each muscle monolayer. After one day of co-culture, neurites were seen growing out of the explant. Reaching their target, they made contact with the myotubes and induced the first contractions after 5 days. After 3 weeks in co-culture, innervated muscle fibers became cross-striated, had well-differentiated neuromuscular junctions and displayed other criteria such as biochemical and pharmacological maturation.

The model was used after 21 days of co-culture when the muscle fibers had a high level of mature neuromuscular junctions.

2.5. Culture treatment and analysis of contraction frequency

Cultures were observed with an inverted microscope (Nikon Diaphot 300) equipped with a camera (DMX 1200 Nikon) for video sequence record and with a motor stage driven by analysis software (Lucia 6.0). Like this, the position of noticeable fields can be recorded and fields can be automatically positioned under lens.

For each experimental point, one well with continuous contraction frequency muscle fiber was selected and the contraction frequency was counted for 30 seconds. The products were then added and the contraction frequency was counted for 30 seconds after 1 minute and 2 hours.

After 48 hours of compound incubation, a visual analysis of cell culture was made in order to verify cell viability.

The results are given as a number of fiber contractions during 30 seconds and as percentage of the contraction frequency compared to the contraction frequency before incubation.

To analyze the results, the following parameters were considered:

- **A 25 % decrease in contraction frequency is not significant**
- **A decrease of frequency contraction comprise between 25 % and 75 % is classify as a slowing down**
- **A decrease higher than 75 % is considered as a blockage of contraction.**

Results are illustrated by representative video recording of muscle contraction (joined CD-Rom).

3 - RESULTS AND CONCLUSION

3.1. Preliminary cytotoxicity determination

Results of the MTT assay are showed in **Table 1**.

The tested concentrations were selected in accordance with the study promoter (see table § 2.2).

It was decided, in agreement with the partner, to test:

- Compound Pentapeptide 099-12: 5mM.

3.2. Modulation of fiber muscle frequency contractions

3.2.1 Control medium

Results are presented in table 2 and illustrated by the video recording "control".

In controls, amongst the 5 observed muscle fibers, only one showed a significant reduction of contraction, by 35 % after 1 minute and by 42 % after 2 hours.

This result shows that some fibers can have a strange behavior. Also, when testing compounds, if a fiber present an answer too different from the other, one or more additional fibers are tested.

3.2.2 Carisoprodol

Carisoprodol is a myorelaxant reference compound that blocks contraction. This compound acts on the motor end-plate.

Results are presented in table 2 and illustrated by the video recording "carisoprodol 0.1mM" and "carisoprodol 0.01mM".

At 0.1mM, Carisoprodol blocked the contractions of the 3 selected fibers after 1 minute and 2 hours.

At 0.01 mM, Carisoprodol slowed down the frequency contraction after 1 minute of incubation. After 2 hours, the fiber recovered the initial frequency contraction.

3.2.3 Compound pentapeptide 099-12

The results are presented in table 6 and are illustrated by the video recording “099-12 5mM”.

The compound at 5mM blocked the contraction of 2 fibers, slowed down the frequency contraction of 1 muscle fiber and has no effect on the last selected fiber. After 2 hours of incubation, 2 fibers were totally blocked, and 2 fibers recovered the initial frequency contraction.

The product at 5mM seems to have a random effect on the frequency of concentration depending on the observed fibers. This means that this concentration could be the lowest concentration having a myorelaxant effect.

In conclusion, pentapeptide 099-12 at 5 mM inhibits partly the frequency contraction after 1 minute and 2 hours.

4 - FIGURES AND TABLES

DO₅₄₀

Pentapeptide 099-12										
mM	0	0.005	0.01	0.1	0.5	1	5	10	15	0
	0.357	0.352	0.310	0.307	0.293	0.294	0.322	0.302	0.327	0.308
	0.361	0.340	0.309	0.323	0.324	0.290	0.340	0.296	0.318	0.296
	0.370	0.355	0.351	0.326	0.343	0.289	0.345	0.326	0.350	0.331
	0.362	0.371	0.341	0.316	0.306	0.309	0.340	0.319	0.331	0.333
	0.371	0.352	0.361	0.343	0.332	0.300	0.300	0.292	0.319	0.335
	0.362	0.323	0.335	0.317	0.303	0.287	0.325	0.289	0.320	0.317
average	0.342	0.349	0.335	0.322	0.317	0.295	0.329	0.304	0.328	
viability (%)	100	102	98	94	93	86	96	89	96	
Observations	+	+	+	+	+	+	+	+	+	

+, normal population

+/-, growth reduction or morphological modifications

-, toxicity

v, vacuoles

g, grains of product

Table 1: Preliminary cytotoxicity determination of the compound

Number of contractions during 30 secondes before product incubation	Product incubation (concentration)	Number of contractions during 30 secondes after 1 min of product incubation (pourcent of contraction)*	Number of contractions during 30 secondes after 2 h of product incubation (pourcent of contraction)*	Observation of cell death after 48 h of product incubation
31	medium control	20 (65 %)	18 (58 %)	no
49		37 (76 %)	45 (92 %)	no
50**		55 (110 %)	53 (106 %)	no
56		55 (98 %)	57 (102 %)	no
56		55 (98 %)	53 (95 %)	no
30	Carisoprodol (10^{-4} M)	3 (10 %)	0 (0 %)	no
46**		0 (0 %)	0 (0 %)	no
25		5 (20 %)	0 (0 %)	no
46**	Carisoprodol (10^{-5} M)	21 (46 %)	42 (91 %)	no
59		38 (64 %)	64 (108 %)	no
51		39 (76 %)	49 (96 %)	no

* percent of contractions compared to the contraction frequency before incubation

** representative video recording

Table 2: frequency modulation of muscle fibers contraction in presence of medium control or **carisoprodol** (myorelaxant compound reference)

Number of contractions during 30 secondes before product incubation	Product incubation (concentration)	Number of contractions during 30 secondes after 1 min of product incubation (pourcent of contraction)*	Number of contractions during 30 secondes after 2 h of product incubation (pourcent of contraction)*	Observation of cell death after 48 h of product incubation
42	Pentapeptide 099-12 (5mM)	33 (79 %)	0 (0 %)	no
42**		13 (31 %)	34 (81 %)	no
41		0 (0 %)	0 (0 %)	no
55		6 (11 %)	42 (76 %)	no

* percent of contractions compared to the contraction frequency before incubation

** representative video recording

Table 3: frequency modulation of muscle fibers contraction in presence of compound pentapeptide 099-12

2. Evaluation, on volunteers, of the anti-wrinkle effect of VIALOX[®] POWDER “Botox-Like” cosmetic treatment

**GROUPE
DERMSCAN**



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EVALUATION, ON VOLUNTEERS, OF THE ANTI-WRINKLE EFFECT OF "BOTOX- LIKE" COSMETIC COMPOUNDS

Report:	#1040226
Estimate:	#0030604-2
Product(s):	1- Product A WSE 104/32 2- Placebo WSE104/34
Form(s) and application(s):	1 and 2 White lyophilisate Applied on half the face
Sponsor:	PENTAPHARM AG Dornacherstrasse 112 CH-4147 Aesch Switzerland
Report date:	June 23, 2004

1 AIMS

1.1. Primary objective(s)

To evidence, on volunteers, the anti-wrinkle effect of each tested product, after 28 days of twice-daily application, using Primos®.

1.2. Secondary objective(s)

Not applicable.

2. METHODS

2.1. Trial period

Estimate:	February 26, 2004
Signed estimate reception:	March 30, 2004
Innocuousness certification reception:	March 30, 2004
Product reception:	April 9, 2004
Subject recruitment:	from April 8 to April 16, 2004
Test:	from April 19, 2004 to May 18, 2004
Data management:	from May 24 to 27, 2004
Statistical analysis:	May 27, 2004
First results by fax:	June 4, 2004

2.2. Experimental plan

This was a simple blind, intra-individual study; each subject was her own control.

2.3. Assessment criteria

2.3.1. Primary criteria

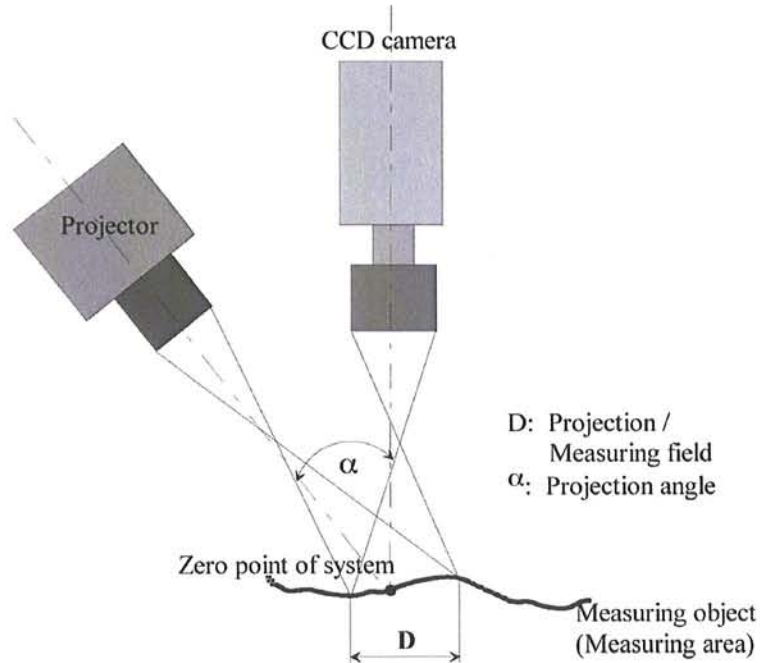
Quantification of the cutaneous relief was done using Primos®.

2.3.2. Secondary criteria

Not applicable.

2.3.3. Principle(s) and measurement instrument(s)

For this technique, parallel stripe patterns are projected, via a system of optics, on the sample (directly on the skin's surface or a skin replica) with successive phase shifts. The resulting images are captured on a digital matrix camera. The analysis of fringe deformations provided a qualitative, as well as quantitative, evaluation of each height profile (3-4).

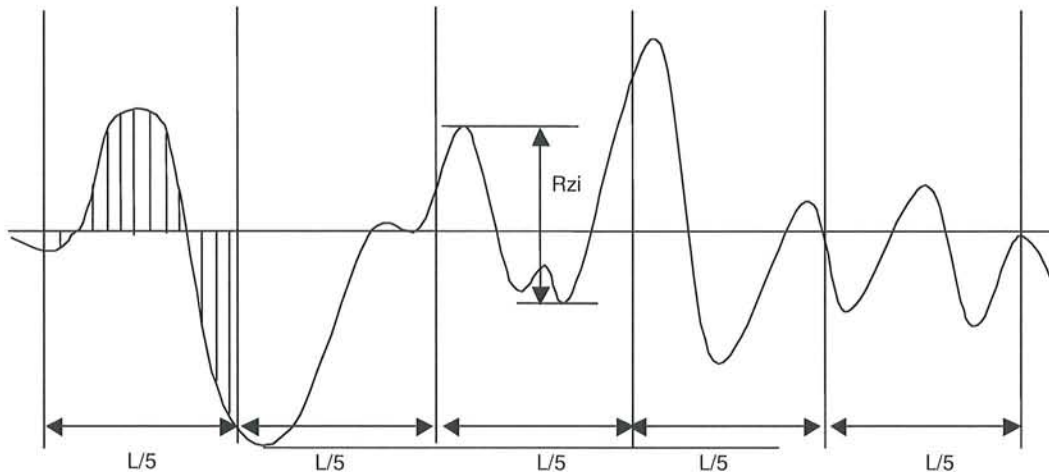


An automatic system of repositioning allows the precise re-identification of the measurement zone.

The acquisition of measurement data is achieved using the PRIMOS[®] software package.

The following cutaneous parameters could be analyzed:

- **Ra**: average roughness (ratio between the integrated surface around the mean value and the length of skin evaluated).
- **Rz**: average relief on five regions of the profile (mean value of these different maxima, obtained on five successive regions of the profile. It reflects local differences).



A decrease of the Ra parameter express a smoothing effect.

A decrease of the Rz parameters express an anti-wrinkle effect.

2.4. Method pertinence

With the development of optical 3D systems, allowing non-tactile measurement done in real time directly on live human skin, it is possible to obtain rapid and precise 3D reconstruction of all kinds of skin surfaces: wrinkles and microrelief, but also ulcers and burn scars, etc. Image processing techniques developed in parallel offer the precise re-identification of skin areas previously subjected to analysis.

2.5. Operational aspect

2.5.1. Trial organization: schedule

On D0

- Subjects came to the laboratory without having applied any product to their face since the previous evening.
- An information sheet was provided to remind them of the study details.
- They read and signed the information and consent forms in duplicate.
- Definition of one crow's feet.
- Image acquisition with PRIMOS® on the defined crow's feet was done.
- Distribution of the products to the volunteers (depending of the randomization) who apply it twice-daily (in the morning and evening on clean skin), for 28 days, on half the face.

Each group of fifteen volunteers test one product reference.

Group one :- One crow's foot is treated with the "product A WSE 104/32"

Group two :- One crow's foot is treated with the "placebo WSE 105/4010"

On D28

- Volunteers came to the laboratory; the last application of the product was done the previous evening.
- New image acquisition with PRIMOS® on the crow's feet defined on D0.
- The volunteers brought back the product.

2.5.2. Adverse Events/Serious Adverse Events

2.5.2.1. Definitions

An Adverse Event is defined as any expression or noxious and not wanted symptom suffered by subjects taking part in biomedical research, whether or not it relates to the tested product(s).

A Serious Adverse Event (SAE) is defined by one of the following criteria:

- death,
- life threatening,
- hospitalization,
- persistent or significant disability or incapacity,
- congenital anomaly,
- overdose,
- cancer,
- other event considered clinically significant by the investigator.

2.5.2.2. Documentation

Any or all Adverse Events related to the tested product (adverse reaction or effect) will be reported in the Case Report Form (CRF) and the study report.

Any or all concomitant treatment will be reported in the CRF and the study report.

Any or all Serious Adverse Events will be reported in the CRF and the study report.

2.5.2.3. Notification

All Serious Adverse Events will be transmitted by fax to the sponsor within 24 hours after knowledge of its occurrence, and then confirmed by mail within 48 hours.

2.5.2.4. Early termination of the study

x Test exit conditions

* In compliance with the Helsinki/Tokyo/Venice declaration and French law dated December 20, 1988 concerning the protection of subjects used in biomedical research, subjects had the right to exit from the study at any time and for any motive.

* The investigator also could have interrupted the treatment prematurely in the case of an intercurrent disease or undesirable effect.

* The sponsor could have demanded that any subject be excluded from the test for major infringements of the protocol, for administrative reasons or any other motive.

Nevertheless, premature removal of a high percentage of subjects from the test could have made the test difficult or impossible to interpret. Consequently, any premature exit without valid motives should have been avoided as much as possible.

Every premature exit must have been classified under one of the following headings:

- Adverse Event occurrence,
- Serious Adverse Event occurrence,
- withdrawal of consent,
- untraceable panelist,
- appearance of exclusion criteria,
- non-adherence to the protocol,
- other reason.

x Replacement conditions

If the premature exit was not related to the test treatment(s), the subject was replaced. Any replacement must have been previously discussed with the trial manager.

2.5.3. Collection and validation of data

The technician responsible for the test added data to subject case report form and to a computerized data base.

Data were then validated by the trial manager.

2.5.4. Trial monitoring visit

A trial monitoring visit may be carried out at sponsor request. It allows the sponsor to verify the study according to the determined protocol.

2.5.5. Quality assurance

The test report is written by the technician responsible for the study and controlled by the trial manager and by a person entitled to exercise the quality control of the study before being sent to the sponsor.

2.6. Subject selection

2.6.1. Inclusion criteria

2.6.1.1. General criteria

- Healthy subjects
- Subjects having given their informed, written consent
- Cooperative subjects, aware of the necessity and duration of controls so that perfect adhesion to the protocol established by the clinical trial center could have been expected

2.6.1.2. Specific criteria

- Sex: female
- Age: between 30 and 60
- Wrinkles on crow's foot

2.6.2. Non-inclusion criteria

- Pregnant or nursing women
- Cutaneous pathology on the test zone(s)
- Ophthalmological pathology.
- Serious or progressive diseases that the investigator judges may interfere with the study
- Medical treatment or product which could modify the cutaneous relief and firmness or termination of this type of treatment within the previous month
- Subjects having had an eye lift or collagen injections
- Excessive use of alcohol or tobacco
- Any medical treatment per os:
 - retinoids and/or immunosuppressives since less than six months,
 - anti cough and/or corticoids since less than four weeks,
 - antihistaminics and/or anti-inflammatories since less than one week,
 - change, start or stop of hormonal treatment or oral contraception since less than six weeks.

2.6.3. Compliance assessment

If the protocol was not respected and if the deviation was minor, the technician responsible for the study warned the subject of the importance of respecting the prescribed protocol. If the subject persisted or if the deviation was major, the subject was declared non-compliant. In this case, the subject was removed from the test for non-compliance.

Under normal conditions of use (application at home), no compliance control could be carried out during the test.

2.6.4. Associated treatment during the study

No systemic treatment likely to modify the skin condition was authorized during the test.

No use of dermatopharmaceutical or cosmetic products other than cleansing products was authorized on the test zones the previous evening or during the study.

2.7. Number of subjects

The study was carried out on 30 subjects (two groups of fifteen volunteers) at the sponsor's request.

2.8. Tested product(s)

2.8.1. Confidentiality procedure

The products supplied by the sponsor were encoded.

2.8.2. Storage

Before the beginning of the study, each product was kept at room temperature in a dedicated air-conditioned room. This room is locked and access controlled.

2.8.3. Reference(s)

- 1- Product A WSE 104/32
- 2- Placebo WSE 104/34
- 3- Solvent WSE 105/4010

2.8.4. Aspect(s)

- 1 and 2 - White lyophilisate
- 3- Colorless solution

2.8.5. Labeling

Example of labeling of each product by the clinical trial center:

DERMSCAN Etude n° Volontaire n°..... Zone :	DERMSCAN Study # Volunteer #..... Zone:
Réf :	Ref.:
En cas d'urgence Tél : 04 72 82 36 59	Emergency telephone number: 04 72 82 36 59
Réf DermScan : Lot n°	DermScan ref.: Batch #:
Conservation : Température Ambiante	Conservation: Ambient Temperature
A utiliser sous stricte surveillance médicale pour essai clinique	For clinical trials: to be used only under strict medical surveillance

2.8.6. Dosage

Twice-daily applications at home (in the morning and evening), for 28 days (see protocol § 2.8.7).

2.8.7. Application site(s) and method(s)

Application site(s): on half the face, on a clean skin.

Application method(s): under normal conditions of use, twice a day, in the morning and evening.

Protocol of use

Vial/Solvent combination should last for at least 1 week application (if applied over the whole face) and is freshly prepared at the beginning of each application week.

Even though 1 set should be more than enough for one week (as the volunteers only apply a product over half the face, or even only the eye area), we recommend to start with a new, freshly prepared set every week.

Each volunteer will need 4 sets (Glass vials + Solvent bottles) per product that they will apply.

1. Removing the upper lid of the glass vial and unscrewing the cap of the plastic bottle
2. The plastic bottle is then inserted into the mouth of the glass vial and screwed into it
3. Squeeze the solution completely from the plastic bottle into the glass vial
4. Shake well until the lyophilizate is completely dissolved
5. Transfer back the product into the plastic bottle by pumping it until the entire content is in the plastic bottle
6. The product is now ready to use!

2.8.8. Product issue

The products were delivered to the volunteers by the technician responsible for the study with an explanation of the application conditions.

2.9. Treatment allocation method

2.9.1. Randomization method

The subject's number was given according to the order of inclusion in the study.
The studied zone was randomized right – left.
One group of 15 volunteers had to test the products "A WSE 104/32" and the other group of 15 volunteers had to test the "placebo WSE 105/4010".

2.9.2. Treatment allocation

Each product was allocated to the subjects according to the above randomization procedure.

2.10. Statistical method(s)

2.10.1. Data analysis

The statistical analysis determined the significance of the results obtained under the effect of each tested product.

The comparison was on the values obtained on the treated zone before and after application at each measurement time.

Data were analyzed with a **paired t-test**. This method tests whether the mean of sample differences between pairs of data is significantly different from the hypothetical mean, zero under the null hypothesis (H_0).

The alternative hypothesis (H_1) was that the average difference was either greater or less than 0 (two-tailed test). Before carrying out a test, a type I error of 5% is chosen (which corresponds to the risk of rejecting a true null hypothesis).

→ If $p > 0.05$, the mean was not different from 0. Data did not show a significant difference between before and after treatment.

→ If $p \leq 0.05$, H_0 was rejected. There was a significant difference between before and after treatment.

2.10.2. Statistical software

The software used was EXCEL 9.0 version 2000.

2.11. Archives

Data will be securely archived digitally and on paper for fifteen years from the date of dispatch of the final report. At the end of this period, the study archives will be destroyed unless otherwise stipulated in writing by the sponsor.

A sample of each tested product will be kept by the laboratory for one year.

3. TEST FOLLOW-UP

- Number of included volunteers: 30
- Number of volunteers who completed the study: 30
- Number of volunteers included in the data analysis: 30
- Trial monitoring visit: no visit took place

4. SUBJECT CHARACTERISTICS

The table below presents the observations concerning the volunteers included in at least one data analysis.

Volunteers testing the "Product A WSE 104/32"

Volunteer	Name	First name	Age	Sex	Phototype	Previous medical or surgical events or medical treatments	Current medical events or treatments
1	LOU	MI	35	F	II	None	None
2	CAR	MO	54	F	III	None	None
3	BLE	FL	44	F	III	Prozac® since 2002; L- Thyroxine® since 1992; Lysanxia® since 2004	None
4	GAL	DA	55	F	II	None	None
5	GOB	MA	43	F	II	None	None
6	BER	SA	37	F	II	Minidril® since 1990	None
7	ASS	NA	49	F	II	None	None
8	REN	JE	53	F	II	Lévothyrox® since 1990	None
9	AGA	PA	46	F	II	Daflon® since 1990; Zolof® since 2003; Lévothyrox® since 2003	None
10	BOU	YA	50	F	II	None	Hept-a-myl®, Celebrex® and Tétratepan® since 04/30/04
11	COL	DA	56	F	II	None	None
12	JAM	CH	37	F	II	None	Efferalgan® from 05/03/04 to 05/10/04
13	SOB	RE	44	F	II	None	None
14	ROL	MA	47	F	II	None	None
15	CAS	MI	55	F	II	None	None
Mean			47	15	F	0	I
Median			47	0	M	13	II
Minimum			35			2	III
Maximum			56			0	IV
SEM			2			0	V
CI 95%			4			0	VI

Volunteers testing the "Placebo WSE 104/34"

Volunteer	Name	First name	Age	Sex	Phototype	Previous medical or surgical events or medical treatments	Current medical events or treatments
16	BOC	HE	58	F	II	Fasomax [®] since 2003; Orocal [®] D3 since 2003	None
17	MON	MI	53	F	II	None	None
18	FOU	MA	55	F	II	None	None
19	GRA	MA	51	F	II	None	None
20	TCH	RO	43	F	III	None	None
21	PLA	CH	50	F	II	Clopixol [®] since 2001	None
22	GAI	CO	54	F	II	None	None
23	MAR	IS	45	F	II	Lévothyrox [®] since 1993; Tardyféron [®] since 2004	None
24	GAN	DA	58	F	II	None	None
25	GRO	MY	37	F	III	None	None
26	FON	EL	53	F	II	Surgestone [®] since 1999	None
27	BAT	FA	52	F	II	None	None
28	LOI	NO	58	F	II	Detensiel [®] since 1994; Estreva [®] since 2004	None
29	ALI	NA	38	F	III	Trinordiol [®] since 2004	None
30	JAL	YV	58	F	II	None	None
Mean			51	15	F	0	I
Median			53	0	M	12	II
Minimum			37			3	III
Maximum			58			0	IV
SEM			2			0	V
CI 95%			4			0	VI

5. RESULTS

5.1. Calculation formulas

The raw variations (Δ) and in percentage ($\Delta\%$) were calculated according to the following formulas:

$$\Delta = (TZ_{ti} - TZ_{t0})$$

$$\Delta \% = \frac{(TZ_{ti} - TZ_{t0})}{TZ_{t0}} \times 100$$

with

TZ: value obtained on the zone treated by the tested product,
t0: before treatment,
ti: after treatment.

For each tested product, the values of the cutaneous relief parameters measured with the Primos[®] on the treated zone, before treatment and after treatment, are presented in the raw values tables in **Appendices 9.1 to 9.4**.

These tables also show the descriptive statistics: means, medians, minima, maxima, standard errors of the means (SEM) and confidence intervals of 95% (CI 95%) of these values.

The tables of variations present, for each product and each time of measurement, the individual values and descriptive statistics, as well as the mean percentage variations and the results of the statistical analysis (p of the Student t-test).

5.2. Anti-wrinkle effect - Primos® measurements

Three parameters are studied:

- **Ra: average roughness** :a decrease characterize a smoothing effect.
- **Rz: average relief on five regions** :a decrease characterize an anti-wrinkle effect.

Synthesis table

Variations of the cutaneous relief parameters between D0 and D28

Product A WSE 104/32

	Kinetic	Δ raw variations (mean \pm SEM)	$\Delta\%$ percentage variations on mean	Significance	% of volunteers with a smoothing effect
Ra (μ m)	D28 - D0	-4.7 \pm 3.6	-11%	NS (p=0.220)	60%
Rz (μ m)	D28 - D0	-16.8 \pm 23.6	-8%	NS (p=0.489)	47%

NS : non significant.

After 28 days of treatment, the product "A WSE 104/32" presented a smoothing effect : decrease of the average roughness (Ra parameter) of 11% in average and decrease average relief on five regions (Rz parameter) of 8%. This effect is respectively observed on 60%and 47% of the volunteer.

Illustrations are presented in appendix 9.1.2.

Placebo WSE 104/34

	Kinetic	Δ raw variations (mean \pm SEM)	$\Delta\%$ percentage variations on mean	Significance	% of volunteers with a smoothing effect
Ra (μ m)	D28 - D0	+4.6 \pm 2.7	+12%	NS (p=0.111)	20%
Rz (μ m)	D28 - D0	+21.0 \pm 14.1	+11%	NS (p=0.157)	40%

NS : non significant.

After 28 days of twice-daily use, the "placebo WSE 104/34" did not have any anti-wrinkle effect on the Ra and Rz parameters. And it even seems to have the contrary effect : average roughness (Ra parameter) and average relief on five regions (Rz parameter) increase (no significantly).

Illustrations are presented in appendix 9.4.2.

6. CONCLUSION AND SIGNATURE(S)

The aim of the study was to evidence, on volunteers, the anti-wrinkle effect of each tested product after 28 days of twice-daily use using Primos®.

Study conditions:

Product reference(s)		1- Product A WSE 104/32 2- Placebo WSE 104/34
Measurement zone(s)		Crow's foot
Number of volunteers included in the data analysis		30
Age	Product A WSE 104/32	47±2 (between 35 and 56)
	Placebo WSE 104/34	51±2 (between 37 and 58)
Specific inclusion criteria		wrinkles on crow's foot
Application for each product		<u>At home</u> : under normal conditions of use, twice daily on a clean face
Protocol		Before/after treatment
Measurement kinetics		D0/D28

Under these study conditions:

- After 28 days of treatment, the product "A WSE 104/32" presented a smoothing effect : decrease of the average roughness (Ra parameter) of 11% in average and decrease average relief on five regions (Rz parameter) of 8%. This effect is respectively observed on 60% and 47% of the volunteer.
- After 28 days of twice-daily use, the "placebo WSE 104/34" did not have any anti-wrinkle effect on the Ra and Rz parameters. And it even seems to have the contrary effect : average roughness (Ra parameter) and average relief on five regions (Rz parameter) increase (no significantly).

June 23, 2004

Scientific Manager
Stéphanie BOUTROY

Analysis Supervisor
Carine KURDIAN

7. CERTIFICATION

Data were obtained using current internal procedures and in compliance with the principles of Good Clinical Practice.

Only the hard copy of the report (green bands) transmitted by DermScan can be considered an attestation and official. Digitally-produced or electronic documents transmitted by DermScan are not protected by an electronic signature, according to Law n°2000-230 dated March 13, 2000 and its applicable decrees. The contents of digitally-produced or electronic documents in no means engage the responsibility of DermScan.

Any modifications are the sole responsibility of the author of the modification, whether he/she is acting for the sponsor or independently. Any partial or total reproduction of this trial report requires prior written agreement from DermScan.

This study was totally performed under the responsibility of the clinical trial center.

8. BIBLIOGRAPHY

8.1. Anti-wrinkle effect

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9. APPENDICES

9.1. Primos® measurements – Product A WSE 104/32

9.1.1. Individual results

Ra (in µm)

Volunteer	D0	D28	Δ(D28-D0)
1	29,5	20,2	-9,3
2	94,8	49,8	-45,0
3	30,3	29,0	-1,3
4	39,2	36,5	-2,7
5	37,5	39,2	1,7
6	36,3	37,3	1,0
7	33,3	45,5	12,1
8	57,2	39,0	-18,2
9	35,4	38,7	3,3
10	72,9	56,0	-16,9
11	30,1	38,7	8,6
12	49,0	42,2	-6,9
13	44,6	42,0	-2,6
14	36,3	35,2	-1,1
15	30,5	38,0	7,4
Mean	43,8	39,1	-4,7
Median	36,3	38,7	-1,3
Minimum	29,5	20,2	-45,0
Maximum	94,8	56,0	12,1
SEM	4,8	2,1	3,6
CI 95%	10,2	4,5	7,8
Δ %			-11
p			0,220
% of volunteers with a smoothing effect			60

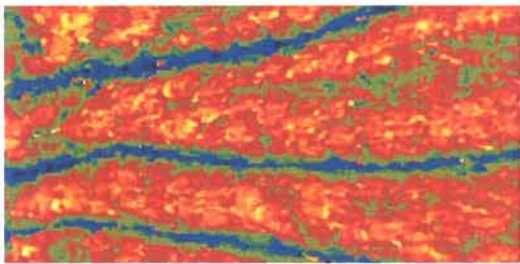
Rz (in µm)

Volunteer	D0	D28	Δ(D28-D0)
1	134,2	116,1	-18,1
2	496,5	255,2	-241,3
3	147,7	159,6	11,9
4	206,0	160,1	-45,8
5	171,2	185,1	14,0
6	187,1	167,5	-19,5
7	183,8	311,6	127,8
8	353,0	182,1	-171,0
9	188,9	212,1	23,3
10	311,9	255,0	-56,9
11	167,7	203,2	35,6
12	253,1	219,0	-34,0
13	208,7	222,5	13,7
14	184,0	210,2	26,2
15	147,8	230,6	82,8
Mean	222,8	206,0	-16,8
Median	187,1	210,2	11,9
Minimum	134,2	116,1	-241,3
Maximum	496,5	311,6	127,8
SEM	25,0	12,3	23,6
CI 95%	53,7	26,4	50,6
Δ %			-8
p			0,489
% of volunteers with a smoothing effect			47

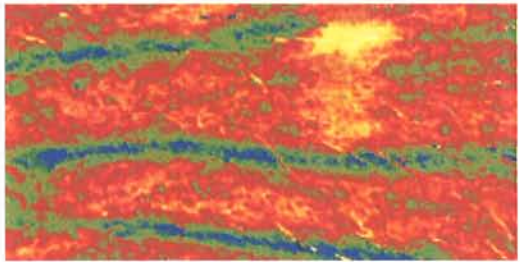
9.1.2. Illustration

Volunteer #2

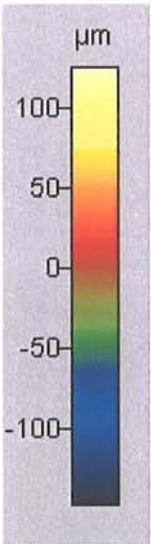
	Ra	Rt
D0	94.8	496.5
D28	49.8	255.2
$\Delta(D28-D0)$	-45	-241.3
$\Delta\%$	-47%	-49%



D0



D28



9.2. Primos® measurements - Placebo WSE 104/34**9.2.1. Individual results****Ra (in µm)**

Volunteer	D0	D28	Δ(D28-D0)
16	21,4	41,9	20,6
17	26,0	32,5	6,5
18	52,8	45,0	-7,9
19	40,4	43,5	3,0
20	25,1	44,2	19,1
21	44,5	45,1	0,6
22	57,9	60,5	2,6
23	33,3	33,5	0,3
24	54,2	40,7	-13,6
25	32,8	36,5	3,7
26	46,3	48,0	1,7
27	40,3	29,6	-10,7
28	41,0	55,8	14,8
29	30,4	46,9	16,5
30	35,8	47,6	11,8
Mean	38,8	43,4	4,6
Median	40,3	44,2	3,0
Minimum	21,4	29,6	-13,6
Maximum	57,9	60,5	20,6
SEM	2,8	2,1	2,7
CI 95%	6,1	4,6	5,8
Δ %			12
p			0,111
% of volunteers with a smoothing effect			20

AV: Aberrant value

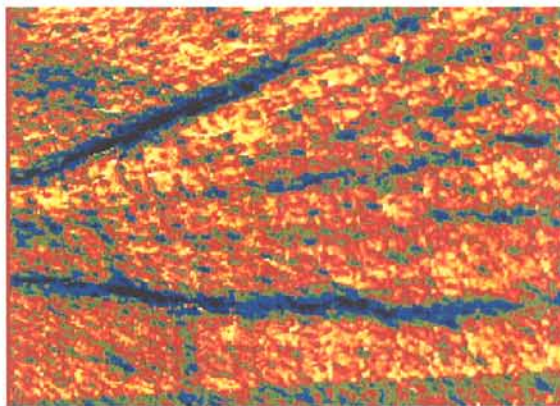
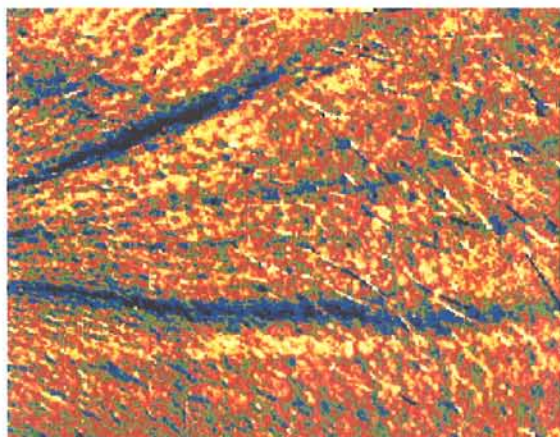
Rz (in µm)

Volunteer	D0	D28	Δ(D28-D0)
16	121,4	214,5	93,1
17	131,6	167,3	35,7
18	266,2	227,8	-38,4
19	201,9	252,7	50,7
20	126,6	209,5	82,9
21	279,1	199,9	-79,2
22	273,4	306,2	32,9
23	171,9	162,3	-9,5
24	259,9	214,0	-46,0
25	173,4	167,0	-6,4
26	236,2	236,3	0,1
27	183,5	165,7	-17,8
28	187,0	290,3	103,4
29	147,4	214,9	67,5
30	158,7	204,8	46,1
Mean	194,5	215,6	21,0
Median	183,5	214,0	32,9
Minimum	121,4	162,3	-79,2
Maximum	279,1	306,2	103,4
SEM	14,3	11,2	14,1
CI 95%	30,7	23,9	30,2
Δ %			11
p			0,157
% of volunteers with a smoothing effect			40

AV: Aberrant value

9.2.2. Illustration

Volunteer #23

**D0****D28**