Polígon Industrial Camí Ral
C/Isaac Peral, 17
08850 Gavá – Barcelona - Spain
Telf. +34 93 638 80 00
fax. +34 93 638 93 93
Internet: www.lipotec.com
e-mail. commercial@lipotec.com

LEUPHASYL®

A NEW PENTAPEPTIDE FOR EXPRESSION WRINKLES

CODE: PD080

Date: June 2005 Revision: 1

A GMP PEPTIDE FOR COSMETIC APPLICATIONS

A NEW SYNTHETIC COSMETIC INGREDIENT

SUMMARY

Lipotec has developed a new peptide to reduce expression wrinkles and that offers two advantages:

- A new and alternative *in vitro* mechanism to fight expression wrinkles
- Additive / Synergistic effect to complement the action of ARGIRELINE®

LEUPHASYL®'s mechanism mimics *in vitro* the natural mechanism of enkephalins: it couples to the enkephalin receptor, on the outside of the nerve cells. When LEUPHASYL® couples to the receptor, a conformational change initiates a cascade inside the neuron that results in a decrease of its excitability: the nerve cell's activity is "turned down" and the release of acetylcholine is modulated. Muscle contraction will be relaxed and therefore, expression wrinkles will be diminished.

GENERAL DESCRIPTION

One of the most striking signs of skin aging is increased wrinkling of the face. This can occur naturally over time and is identified by certain biochemical, histological and physiological changes that are enhanced by environmental exposure. There are other secondary factors that can cause characteristic folds, furrows and creases of the face. These include the constant pull of gravity, frequent and constant positional pressure of the skin of the face (e.g. during sleep) or repeated facial movements caused by the contraction of the muscles of facial expression. In any case and independently of the ultimate physiological pathway, the molecular mechanism involved in face aging is directly related to changes in the conformation of the collagen triple helix, degradation of the elastin polypeptides and certain disorder in the packing of the lipidic matrix of the skin.

It has been clearly established that these conformational changes and the disturbance of the perfect packing of the lipid matrix can be significantly avoided by modulating muscle contraction.

Muscles are contracted when they receive acetylcholine released from inside a vesicle. LEUPHASYL is an enkephalin modified for enhanced stability that provides a modulation of acetylcholine release from neuron cell cultures. This may attenuate muscle contraction, preventing the formation of lines and wrinkles.

PROPERTIES AND APPLICATIONS

- LEUPHASYL® reduces the depth of wrinkles on the face caused by the contraction of muscles of facial expression, especially in the forehead and around the eyes.

- LEUPHASYL® targets in vitro the wrinkle-formation mechanism of expression wrinkles in a new way, offering an alternative to peptides like Argireline®.

LEUPHASYL® can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where removal of the deep lines or wrinkles in the forehead or around the eyes area is desired.

TECHNICAL INFORMATION

PRODUCT SPECIFICATIONS

LEUPHASYL® Solution	
Code:	PD080
INCI name:	Water, Glycerin, Pentapeptide-3 (proposed)
Appearance:	Translucent solution
Contents:	0.05 % LEUPHASYL® powder
Preservative:	0.5 % Caprylyl Glycol

The synthesis of LEUPHASYL® is carried out at our factory in Gavà following cGMP guidelines and involves a final freeze-drying step. Freeze-dried products are commonly obtained as a polymorphous crystalline powder, which means that locally some aggregates and differences in crystal size may appear. This polymorphism is not associated to chemical differences and extensive work performed by the analytical department has ensured the homogeneity of the product.

PROCESSING AND DOSAGE

LEUPHASYL® is presented as **LEUPHASYL® Solution**, an aqueous solution containing 0.5 g/L of the peptide powder. It can be incorporated at the final stage of the manufacturing product, provided the temperature is below 40 °C. Taking into consideration the concentration of peptide in **LEUPHASYL® Solution**, it is recommended that 3 to 10% of the solution is present in the final formulation in order to obtain significant anti-wrinkle activity.

STORAGE AND SHELF LIFE

LEUPHASYL® Solution must be kept in a cool, dark and clean place to ensure a shelf life of at least twelve months.

For long term storage it is recommended to store both forms at 4 ºC which extends shelf life to at least eighteen months. In rare cases, refrigerated storage of **LEUPHASYL® Solution** can cause precipitation of the preservative. This does not affect the integrity of the product.

SAFETY

The toxicological profile of LEUPHASYL® for cosmetic purposes was assessed only "in vitro" or on a panel of human volunteers (in Spain). A full toxicological report and a summary of all the safety tests performed are available on request.

All tests were performed using solutions of LEUPHASYL® at the desired concentrations.

In vitro tests

Citotoxicity test on human dermal fibroblasts
No signs of citotoxicity were observed.

Citotoxicity test on human epidermal keratinocytes
The results showed no signs of citotoxicity at the concentrations assayed.

Genotoxicity test (Ames test)
The results showed no genotoxicity under the conditions assayed.

Ocular Irritation (HET-CAM test)
The product is potentially not irritating for the eyes.

In vivo tests

Acute oral toxicity test
Analysis design allowed to conclude that DL_{50}>2500 mg/Kg body weight in rats and therefore LEUPHASYL® shows no acute oral toxicity at the dosage tested. This test is compulsory for any new chemical entity according to the Dangerous Substances Directive 67/548/EC.

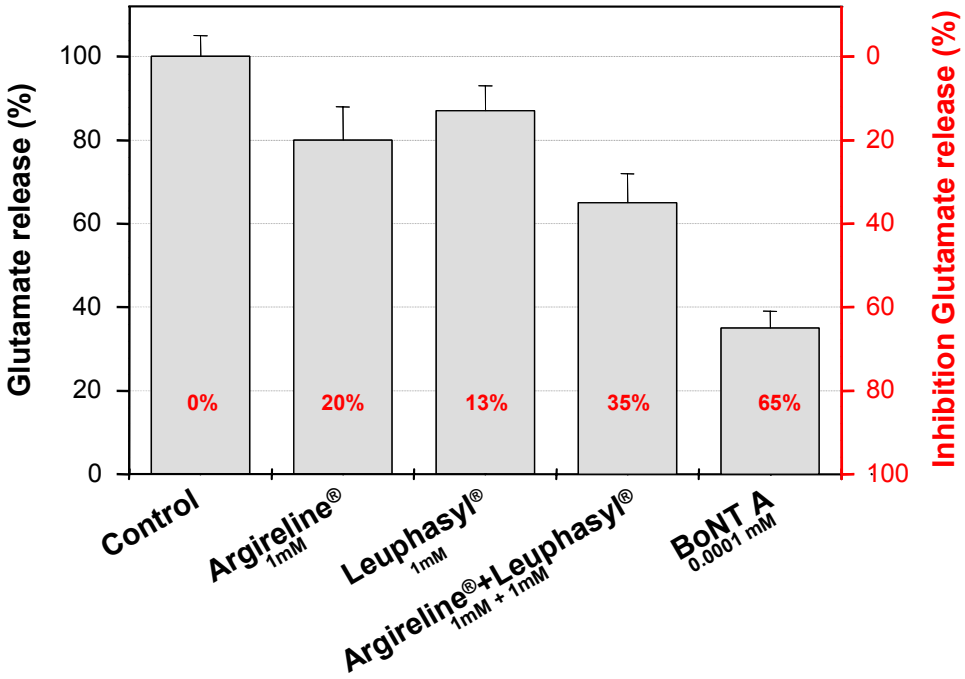
Skin irritation (Patch Test)
The test was performed on 10 human volunteers, aged 18 to 70, both sexes, phototype Fitzpatrick I to V. LEUPHASYL® Solution (20µl) was applied on the back, under an occlusive patch for 48 hours +/- 5 hours. Skin examination was performed visually under standard "daylight" source, 15 minutes (or more if some redness appeared after patch removal), then 24 hours +/- 2 hours after patch removal.
The results of the individual daily irritation score (Idis) and the mean daily irritation score (Mdis) were 0, and the conclusion was that LEUPHASYL® Solution has a very good skin compatibility

EFFICACY DATA

In vitro tests

Modulation of glutamate release in a neuron cell culture

A cell culture of neurons was incubated with tritiated glutamine during 3h in order to load them with radiolabelled glutamate. Glutamate is the most common neurotransmitter in the brain and its release is used as an estimate for acetylcholine release. The release of glutamate from the neurons is measured in order to compare the *in vitro* activities of all the peptides.



Both peptides show a synergistic effect *in vitro*, which means their mechanisms are independent and their effects can be added!

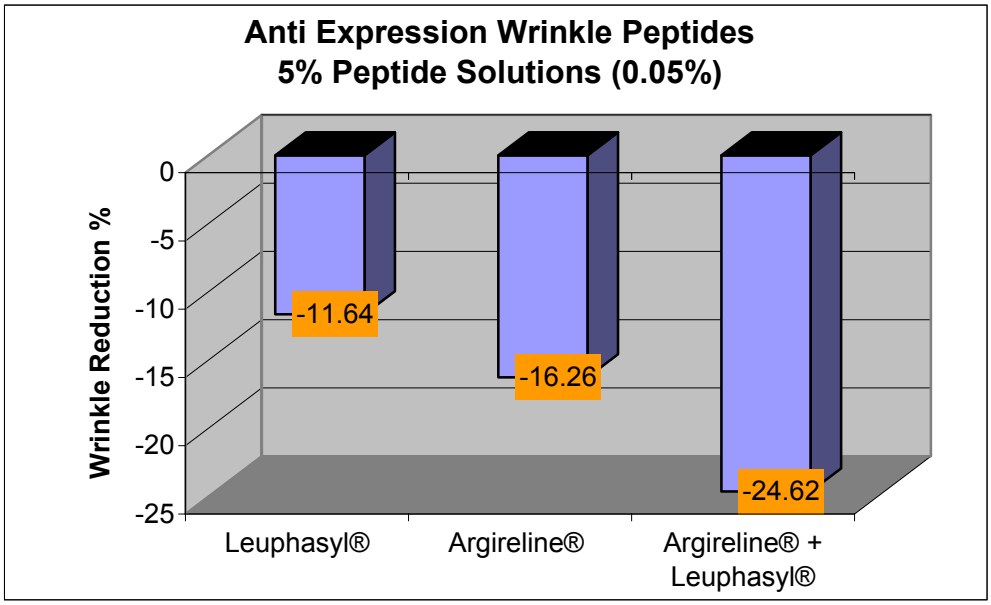
In vivo test

In order to prove an alternative mechanism to Lipotec's other expression anti-wrinkle, ARGIRELINE®, parallel tests were performed in order to compare both peptides. Tests were performed using a placebo cream.

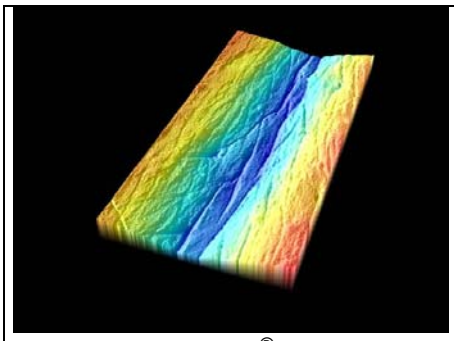
Efficacy of LEUPHASYL® as an anti-wrinkle agent was evaluated by taking silicon imprints of the wrinkles around the eyes of healthy volunteers. These wrinkles are expression wrinkles. A cream containing 5% LEUPHASYL® SOLUTION (0.05%) was applied twice daily around the eyes of 14 volunteers, aged 39 to 64, for 28 days. A **decrease of 11.64%** was accomplished, with maximum values up to –23.55%.

A cream containing 5% ARGIRELINE® SOLUTION (0.05%) was applied twice daily around the eyes of 14 volunteers, aged 39 to 63, for 28 days. A **decrease of 16.26%** was accomplished, with maximum values up to –31.80%.

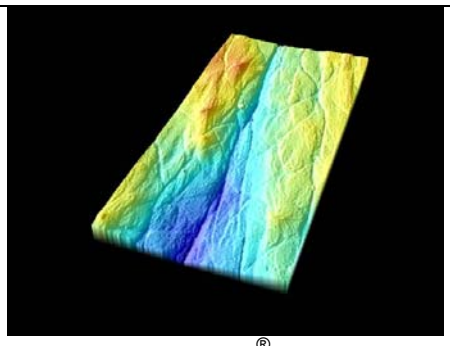
A third test was performed using a combination of both actives in order to evaluate a possible synergy or additive effect. A cream containing 5% ARGIRELINE® SOLUTION (0.05%) + 5% LEUPHASYL® SOLUTION (0.05%) was applied twice daily around the eyes of 15 volunteers, aged 39 to 63, for 28 days. A **decrease of 24.62%** was accomplished, with maximum values up to –46.53%. Both peptides show additive effects due to their complementary mechanisms.



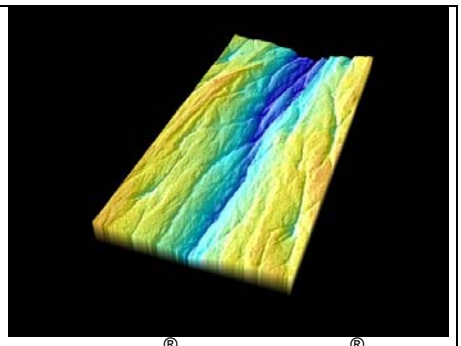
The following are representative images of the silicon replicas:



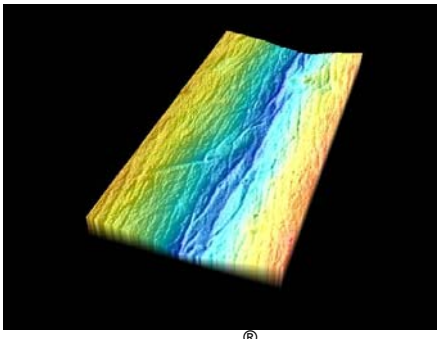
LEUPHASYL® Day 0



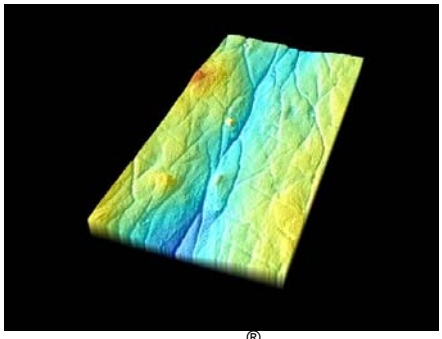
ARGIRELINE® Day 0



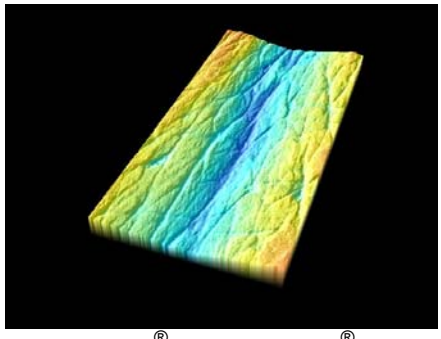
LEUPHASYL® + ARGIRELINE® Day 0



LEUPHASYL® Day 28



ARGIRELINE® Day 28



LEUPHASYL® + ARGIRELINE® Day 28

Dark blue colour indicates maximum depth – bright red colour indicates max height

GENERAL PRODUCT INFORMATION

Trade name	LEUPHASYL® SOLUTION
Product code	PD080

INGREDIENTS

INCI name	CAS No	EINECS No
WATER (AQUA)	7732-18-5	231-791-2
GLYCERIN	56-81-5	200-289-5
PENTAPEPTIDE-3 (proposed)	64963-01-5	-
CAPRYLYL GLYCOL	1117-86-8	214-254-7