

ARGIRELINE®

A New Anti-Wrinkle Peptide (Acetyl Hexapeptide-3)

Cosmetic Application

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

Use Levels

As a result of findings in the various efficacy tests that were conducted, it was found that the recommended dosage of Argireline is 5%.

Storage

Argireline is supplied in solution form at a concentration of 0.05%. It has been shown to have good stability in this form, but is recommended to be stored under refrigeration for maximal long term stability.

Product Specifications

Argireline solution

Code:	PD010
INCI name:	Water, Acetyl Hexapeptide-3
Appearance:	Transparent solution
% Argireline:	0.05%

Please contact us for complete study results, further technical information, and samples.

Manufactured by

LIPOTEC, S.A.
(Barcelona, SPAIN)

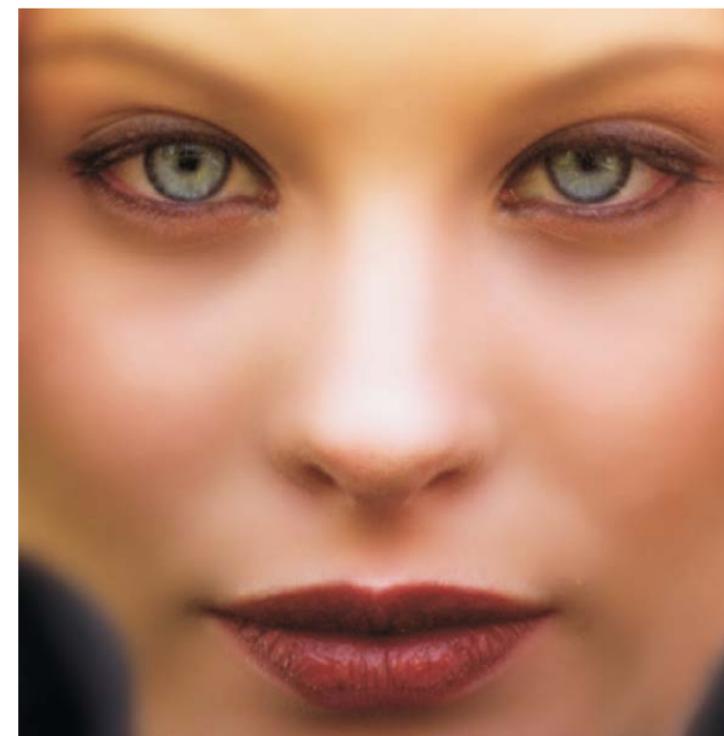
Exclusive North American Distributor


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IN VIVO TESTING

Determination of efficacy against skin wrinkling

In an independent test conducted by Advancell, S.A. (Spain), a study of the effect of Argireline on the elasticity of the skin around the eyes was performed. Using silicone skin replicas and confocal microscopic analysis, the researchers measured changes in the depth of skin wrinkling.

The skin replicas below (see Figs. 3, 4) show the improvement in skin smoothness at 15 and 30 days post-Argireline treatment, compared with the results obtained without the incorporation of Argireline into the test cream.

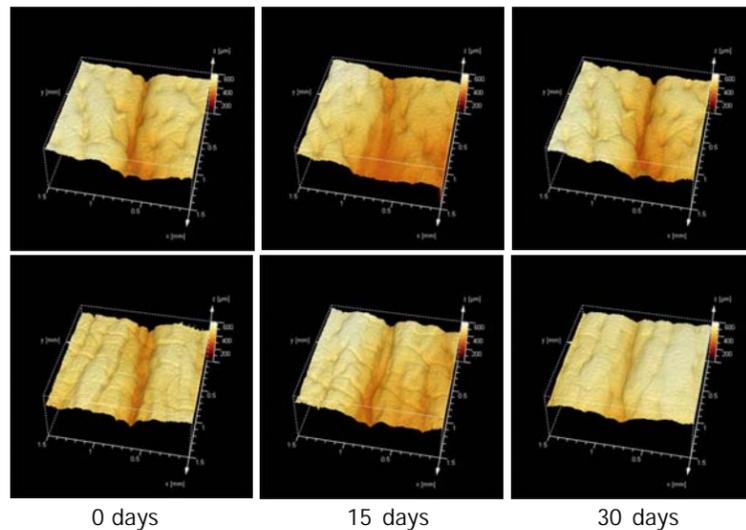


Fig. 3. Silicone replicas of skin that was untreated (top row) and treated with an Argireline-containing cream formulation.

The researchers concluded that Argireline reduced the depth of wrinkles up to 17% after 15 days and 27% following 30 days of treatment.

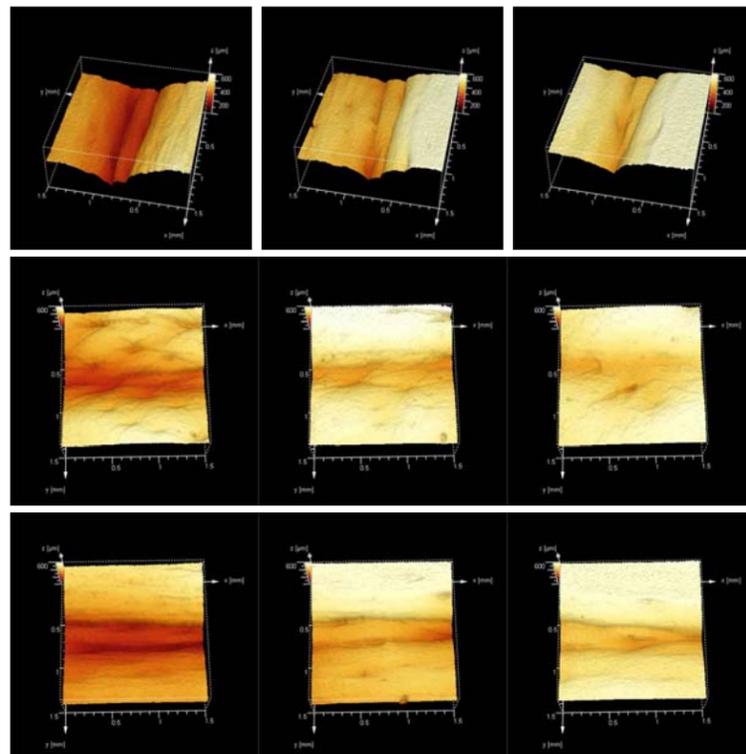


Fig. 4. Additional replicas of skin that was treated with an Argireline-containing cream formulation at 0, 15, and 30 days.

ARGIRELINE®

A New Anti-Wrinkle Peptide (Acetyl Hexapeptide-3)

Argireline: Inhibits Wrinkle Formation

Argireline® is a unique new anti-wrinkle peptide that has been shown in significant testing to be effective against the development of skin wrinkling. *In vitro* methodologies have demonstrated that Argireline inhibits both the formation of the SNARE complex as well as the release of catecholamines. These specific inhibitions confer anti-aging activity on this novel peptide, as these biological processes closely relate to the basic biochemical mechanisms of wrinkle formation.

Controlled studies of Argireline demonstrated that it can reduce the depth of wrinkles on the face caused by the contraction of muscles of facial expression, especially in the forehead and around the eyes. Furthermore, it can prevent apparent skin aging induced by repeated facial movements caused by excessive catecholamine release.

Skin topography analysis performed on healthy female volunteers confirmed the validation of the proposed biochemical mechanism of action. These studies are available upon request.

Background

Modern drug discovery has reached a new pinnacle of achievement in the past few years with the impact of **combinatorial chemistry** and **solid phase peptide synthesis** – two disciplines which enable the rapid exploration and determination of chemical structure-activity relationships (SAR's). They can then be used to identify and create new active ingredients for subsequent commercial production.

With the increasing accessibility of these advanced technologies, progressively sophisticated (and functional) principles are becoming available to cosmetic formulators. For new product developers, this payoff is especially welcome in the area of anti-wrinkling skin care products.

A case in point: The application of combinatorial chemistry and peptide solid phase synthesis has led to the discovery of an acetyl hexapeptide, Argireline, that has been demonstrated in clinical studies to be effective against the visible signs of aging.

Causes of Skin Wrinkling

Skin wrinkling associated with aging is caused by many biochemical, histological and physiological changes that are amplified by environmental exposure and other secondary factors. Beyond the physiological pathways, molecular mechanisms involved in facial aging include changes in the conformation of the collagen triple helix, degradation of the elastin polypeptides and problems relating to the packing of the lipidic matrix of the skin.

It has been clearly established in recent publications that these conformational changes and the disturbance of the packing of the lipid matrix can be significantly reduced by inhibiting the formation of the SNARE complex.

The SNARE complex is a core of membrane proteins that mediate neuronal exocytosis (A. Ferrer Montiel et. al., *The Journal of Biological Chemistry*, 272, 2634-2638 (1997)). Inhibition of this complex by means of short synthetic peptides can decrease the formation of facial

wrinkles, and thereby the appearance of aging of the skin. It is also known that the overproduction and release of catecholamines encourages the formation of wrinkles and fine lines (A. Ferrer Montiel, *FEBS Letters*, 435,84-88 (1998)).

The Technology

Argireline's specific sequence was discovered within a combinatorial library of hexapeptides developed by Lipotec, SA (Barcelona, Spain). Several proprietary processes led to the identification of this particular acetylated hexapeptide, which was shown to inhibit the SNARE complex formation and to also inhibit catecholamine release in high throughput screening conducted on chromaffin cells.

Once identified, the biologically active hexapeptide was synthesized by solid phase peptide synthesis using the Fmoc/tBu protocol. The peptide was purified and characterized by amino acid analysis, mass spectroscopy and NMR. Homogeneity of the peptide was assessed by HPLC and capillary electrophoresis.

In order to determine the inhibition of the SNARE complex, chromaffin cell cultures were maintained in monolayer cultures at a density of 625,000 cells/cm². The ternary SNARE Complex was immunoprecipitated and incubated with and without (control) Argireline and other related peptides. Immuno-complexes were analyzed using SDS-PAGE (4-20%) under non-reducing conditions and immunoblotted with an anti-syntaxin mAb.

Argireline was found to inhibit vesicle docking by preventing the formation of the essential ternary SNARE Complex. Inhibition of noradrenaline and adrenaline release was also demonstrated in a second *in vitro* study supporting the anti-wrinkle activity observed in a thirty (30) day clinical study.

In vivo tests further demonstrated the benefits of Argireline. Skin topography analysis (for measuring the effectiveness of an O/W emulsion containing 5% Argireline) was performed using silicon replicas from around the eyes of ten healthy female volunteers. Replicas were obtained at 0, 15 and 30 days of a twice-a-day treatment regimen. Analyses of the imprints were performed by confocal laser scanning microscopy to assess the evolution of the skin surface before and after treatment. Skin topography images from the three dimensional reconstruction of optical sections are available upon request. It was observed that the severity of wrinkles around the eyes decreased up to 17% after 15 days of treatment and up to 27% after 30 days of treatment, substantiating the proposed biochemical mechanism hypothesis.

Argireline Efficacy Testing

The anti-wrinkle effect of Argireline was ascertained in two different *in vitro* tests directly related to the formation of wrinkles in the epidermis as well as a separate *in vivo* test performed on healthy human volunteers.

IN VITRO TESTING

Cytotoxicity test on human dermal fibroblasts

The test was conducted on human dermal fibroblasts at concentrations between 10 µg/mL and 1 mg/mL with a cell density of 21,000 cell/cm². No signs of cytotoxicity were observed.

Cytotoxicity test on human epidermal keratinocytes

The test was carried out on human epidermal keratinocytes at concentrations between 10 µg/mL and 1 mg/mL with a keratinocyte density of 15,000 cell/cm². The results showed no signs of cytotoxicity at the concentrations assayed.

SNARE complex modulation in chromaffin cells

This test evaluates the inhibition of the SNARE complex formed by peptides derived from the N-terminus of SNAP-25 (synaptosome-associated protein of 25kDa). Argireline modulates SNARE complex formation at concentrations in the mM range (see Fig. 1).

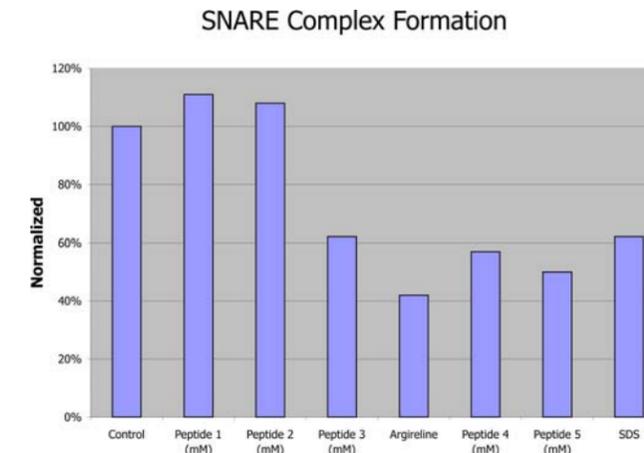


Fig. 1. Modulation of SNARE complex formation by analogues of the N-terminus of SNAP-25.

Chromaffin cells were prepared from bovine adrenal glands by collagenase digestion and further separated from erythrocytes and other impurities by centrifugation gradient. Cells were maintained in monolayer cultures at a density of 625,000 cells/cm².

The ternary SNARE complex was immunoprecipitated from rat brain synaptosomes and incubated with Argireline and other related peptides, or without them (as a control). Immunocomplexes were analyzed using SDS-PAGE (4 – 20 %) under non-reducing conditions and immunoblotted with an anti-syntaxin mAb.

Argireline proved to modulate vesicle docking by attenuating the formation of the essential ternary SNARE complex.

Modulation of catecholamine release in chromaffin cells

Inhibition of the release of catecholamines was determined by monitoring the neurotransmitters adrenaline and noradrenaline. Chromaffin cells were incubated with tritiated noradrenaline/adrenaline and Argireline. The release of catecholamines, as well as the total cell content, was determined by liquid scintillation counting. The significant modulation of both neurotransmitters at nM concentrations of Argireline is a clear indicator of the potent anti-wrinkle activity of this hexapeptide (see Fig. 2).

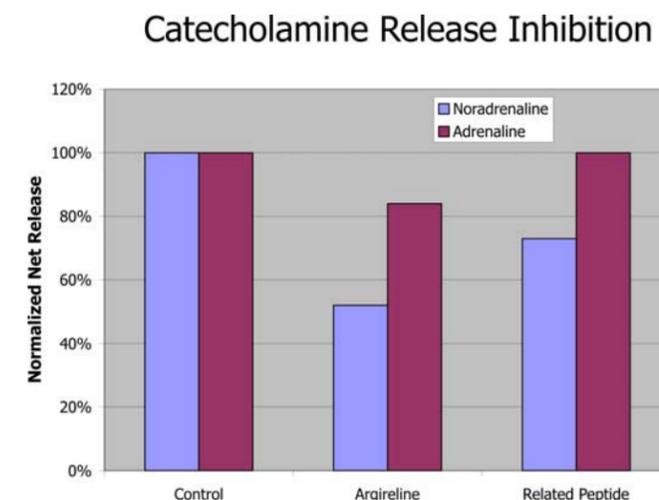


Fig. 2. Modulation of catecholamine release