Technical Report

Product

ARGIRELOX™ *peptide*

Date

March 2014

Revision

0

Lipotec

Pol. Ind. Camí Ral C/ Isaac Peral, 17
08850 Gavà Barcelona (Spain)
Tel. +34 93 638 80 00
www.lipotec.com
commercial@lipotec.com

argirelox™ peptide

Aimtec® peptides and synthetic molecules

Contents

Enhanced and prolonged youth

Looking older than the real chronological age is not a common wish. However, the passing of the years translates into several changes, the face being a specially evident target for them. Facial **wrinkles are a common visual sign of aging** that is not normally accepted nor wanted, whose presence can be caused by external and internal factors.

Expression lines are one of such undesired wrinkles that can betray chronological age and appear even sooner than expected. They are the result of daily repeated facial movements that imply multiple **muscle contractions**.

Injections with Botulinum Toxin Type A (BoNT-A) are a widely used option to effectively attenuate expression wrinkles, like frown lines and the ones located in the crow's feet area, by paralyzing the muscle. A single dose provides a **visible smoothing benefit within a short time** (few days normally) and it can **last for weeks and even months** depending on the case. That is the reason why such treatment is so common nowadays even though its noticeable effect eventually fades, leading to the possible reappearance of expression lines. The recommended time between two BoNT-A injections is 6 months, as the immune system could cause rejection. Thus, individuals need to wait until they can receive the next session to maintain the anti-wrinkle effect.

Cosmetics are the **perfect topical complement** to such injections because they offer the possibility to enhance BoNT-A anti-wrinkle benefits as well as prolong them until the next dose can be injected. Acting on the key steps that **attenuate muscle contraction**, topical ingredients represent a non-invasive treatment that can reduce expression lines and be **applied every day** to maintain BoNT-A smoothing effects on the skin.

The anti-expression lines effect of BoNT-A injections can be enhanced and prolonged by topical cosmetics applied between sessions.

The development of expression wrinkles

Distinguishing the diverse types of aging brings more precision to the treatment, as each type responds differently depending on the origin. On one hand, cutaneous chronological aging (or intrinsic) is characterized by a thin skin prone to atrophy, xerosis, sagginess, hypopigmentation and the appearance of fine lines. On the other hand, long-term sun exposure (photoaging) typically results in deeper wrinkles, creases and furrows, xerosis and hyperpigmentation.

Additionally, wrinkles can appear due to the **repeated contractions** exerted at the same anatomical sites as a result of daily facial expression and movements when talking, smiling, drinking or smoking [1, 2]. These fine lines and wrinkles are known as **expression** (dynamic) **wrinkles**. Frown lines, glabellar lines and crow's feet wrinkles are representative examples, consequence of the **interactions between neuronal and muscle cells** in a process known as neuronal exocytosis.

Initially, the neuron is at its **resting potential** surrounded by a high Na^+ and low K^+ concentration environment. Its activation takes place when after receiving a signal, **Na^+ channels open**. This fact implies a massive entry of sodium ions, that positively charge the inside and cause membrane potential to rise fast. After a maintained period of Na^+ ions entry, these **channels close and inactivate** in order to return the neuron to its resting potential. The opening of K^+ channels helps to remove positive charges from the inside and consequently decreases membrane potential.

At the end of the axon from which the impulse is coming, the membrane depolarizes, gated Calcium channels open, and calcium ions (Ca^{2+}) are allowed to enter the cell. Interesting to mention that enkephalins are endogenous compounds that can indirectly close Ca^{2+} channels, avoiding the following cascade of events related to muscle contraction.

The Vesicle-Associated Membrane Protein (**VAMP**), the membrane-associated protein **syntaxin** and SyNaptosomal Associated Protein 25 (**SNAP-25**) are collectively called SNAP Receptors (SNARE) proteins [3]. They form a crucial ternary structure known as **SNARE complex** that acts as a cellular hook, capturing vesicles containing the Acetylcholine (ACh) neurotransmitter. When Ca^{2+} ions enter the pre-synaptic terminal, the vesicles with ACh are induced to fuse with the neuronal membrane [3, 4]. Muscle contraction occurs when **ACh traverses the synaptic space** and binds to its receptors located in the post-synaptic membrane of **muscle cells**.

Two key events are needed for muscle contraction and expression wrinkle development: entry of Ca^{2+} ions inside the neuron and the formation of the SNARE complex.

Injections to attenuate expression lines

Beauty treatments to improve facial appearance and erase the signs of aging are trendy and really common nowadays. Surgical operations, nonsurgical procedures and cosmetics are very well-known possibilities, acting at different levels. Surgical procedures are one of the most extreme options to hide facial aging, but they are normally highly invasive and expensive, and present potential adverse effects. Nonsurgical procedures are a less invasive option that basically includes injections, laser treatments and abrasion processes.

Botulinum Toxin Type A treatment is a popular **nonsurgical cosmetic procedure** performed since its approval by the Food and Drug Administration (FDA) to temporarily **smooth moderate to severe frown lines** and, after more than a decade, **crow's feet** as well (2013). A trained and qualified physician is needed to inject this protein complex, produced by the bacterium *Clostridium botulinum*. The recommended time between sessions is 6 months minimum.

A small sterile dose of this purified toxin injected on the skin blocks the release of ACh by nerve cells as it **cleaves the SNAP-25 protein**, thereby interfering with vesicle docking and exocytosis. This action prevents the nerve impulses and ACh release at the neuromuscular junction that cause muscle contractions. The **muscles** are then **paralyzed** and the existing frown lines and crow's feet are smoothed. Its effects are visible within a short period of time (4-5 days normally) and they can last for weeks or months (depending on the area).

According to the International Society of Aesthetic Plastic Surgeons, more than 3 million nonsurgical procedures with BoNT-A were performed around the world in 2011, accounting for 38.1% of the total. This datum confirms that the concern about wrinkles and fine lines is extended and that fillers are highly demanded.

In the US, the BoNT-A procedures **increased by 8%** in 2012 compared to the previous year (according to the 2012 report from the American Society of Plastic Surgeons). Thus, it seems that such injections are the **stars when talking about plumping and filling expression wrinkles**, a trend that it is expected to continue in the coming years.

BoNT-A injections are a widely extended treatment to attenuate expression wrinkles, by paralyzing facial muscles.

Complementing smoothing benefits

Topical cosmetics can manage to **enhance and complement** the smoothing effect on the skin of **BoNT-A injections** through several mechanisms. Skin care ingredients reduce the formation of the SNARE complex and the ACh release, providing an anti-wrinkle effect and prolonging the benefit of the toxin over time. Acetyl Hexapeptide-8 and Pentapeptide-18 are two clear examples of anti-expression wrinkle ingredients for skin care.

Widely known, **Acetyl Hexapeptide-8** is the first anti-expression wrinkle peptide. This compound is a **replica of the N-terminal end of SNAP-25** and competes with this natural protein for a position in the **SNARE complex**, modulating its formation without breaking any of its components. If such complex is **destabilized**, the vesicles cannot release ACh efficiently and the **muscle is relaxed**. This topical ingredient targets the same protein complex as the famous toxin but in a different way, without paralyzing the muscle.

Pentapeptide-18 is a modified enkephalin that **modulates ACh release** from neuron cells. It mimics this family of peptides that endogenously inhibit neuronal activity by joining enkephalin receptors outside nerve cells, which are coupled to G proteins. This interaction results in the release of such G protein subunits that, in turn, close Ca^{2+} channels. Thus, a decrease of the neuronal excitability is induced, and **vesicle fusion and ACh release** towards the synapse is **prevented**. **Muscle contraction** is therefore **attenuated**.

Applied between BoNT-A sessions, topical ingredients are able to maintain the smoothing effect of the injection and prolong the skin benefits, by acting on crucial muscle-contraction steps that cause expression wrinkles to appear.

The formation of the SNARE complex and ACh release are diminished by topical ingredients to prolong BoNT-A smoothing effects.

ARGIRELOX™ *peptide*, while waiting for the next injection

ARGIRELOX™ *peptide* is an effective ingredient to fight expression wrinkles, complementing and prolonging the smoothing benefits of Botulinum Toxin A injections. It contains Acetyl Hexapeptide-8 and Pentapeptide-18, which act on the formation of the SNARE complex and on the closure of calcium channels respectively, finally reducing muscle contractions. These complementary mechanisms help to potentiate and maintain the anti-wrinkle effect.

The efficacy of ARGIRELOX™ *peptide* as an anti-expression wrinkle agent was proved in *in vitro* and *in vivo* studies. Its **peptides** showed a **synergistic effect** *in vitro,* where glutamate release inhibition was higher than the sum of their individual effects.

In vivo, its efficacy was demonstrated on volunteers **after** their treatment with **BoNT-A injections**. Still when the line-smoothing effect of the toxin faded, the daily application of ARGIRELOX™ *peptide* helped to **maintain such benefit** on the skin and provided a **long-lasting effect** even 6 months after the injection.

ARGIRELOX™ *peptide* was designed to **prolong** the anti-wrinkle effect of BoNT-A treatments **between sessions**, allowing the benefits to be visible and sustained for longer.

ARGIRELOX™ *peptide* fights expression lines effectively and prolongs BoNT-A effects between sessions.

In vitro efficacy

MODULATION OF GLUTAMATE RELEASE

Inhibition of glutamate release by depolarized neuron cells is a validated cell assay for measuring the potential activity of compounds on the inhibition of neuronal exocytosis. The K^+-induced depolarization of hippocampal cultures in the presence of extracellular calcium ions results in the release of glutamate, which is the most abundant excitatory neurotransmitter in the nervous system.

Primary neuron cells were incubated with L-[^{3}H]-glutamine to charge them with L-[^{3}H]-glutamate. Afterwards, the excess of L-[^{3}H]-glutamine was rinsed off and they were incubated with Acetyl Hexapeptide-8, Pentapeptide-18 or both. The release of L-[^{3}H]-glutamate was carried out by depolarization in a physiologic buffer.

The culture media was collected and the quantity of L-[^{3}H]-glutamate was determined by a scintillation counter. The results were normalized regarding the release of L-[^{3}H]-glutamate in absence of the test items (control) and corrected from the basal release in absence of calcium.

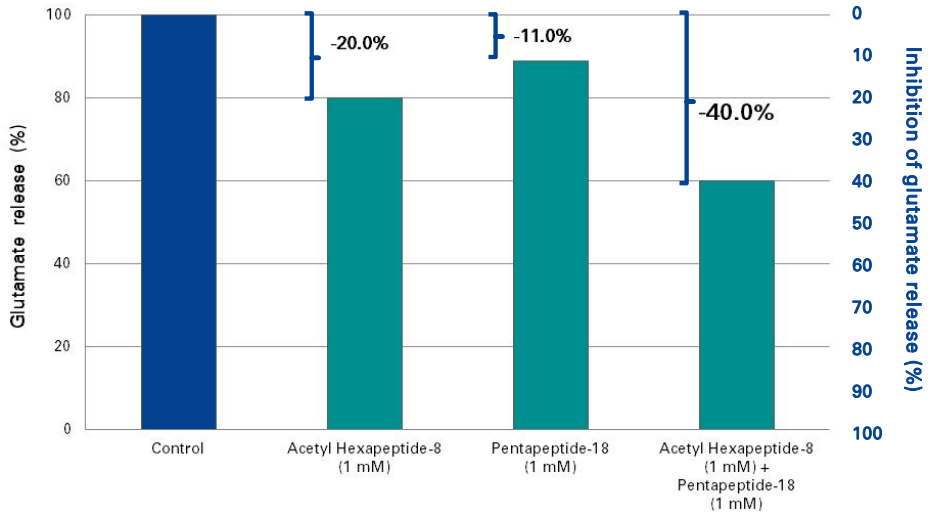

Fig. 1. Inhibition of glutamate release due to the different treatments.

Acetyl Hexapeptide-8 and Pentapeptide-18 induced a -20.0% and -11.0% decrease on glutamate release respectively, while their combination caused a -40.0% reduction, acting **synergistically**.

ARGIRELOX™ *peptide* ingredients offer a complementary effect in reducing glutamate release.

In vivo efficacy

ANTI-WRINKLE EFFECT BETWEEN INJECTIONS

The objective of this study was to evaluate the anti-wrinkle effect of ARGIRELOX™ *peptide* after a Botulinum Toxin A treatment (injection).

A panel of 22 Caucasian females (51 years on average) received 50 UI of the toxin in the periorbital (crow's feet) and frontal region.

After the injection, volunteers applied either the active formulation with 10% ARGIRELOX™ *peptide solution* or a placebo formulation (control treatment) twice a day for 6 months.

Skin silicon replicas were obtained before the injection and at different times during the treatments. The skin relief was evaluated by confocal profilometry, quantifying the reduction of the average surface roughness (Ra) versus base line.

Macroscopic photographs were also taken at base line and after 2, 4 and 6 months.

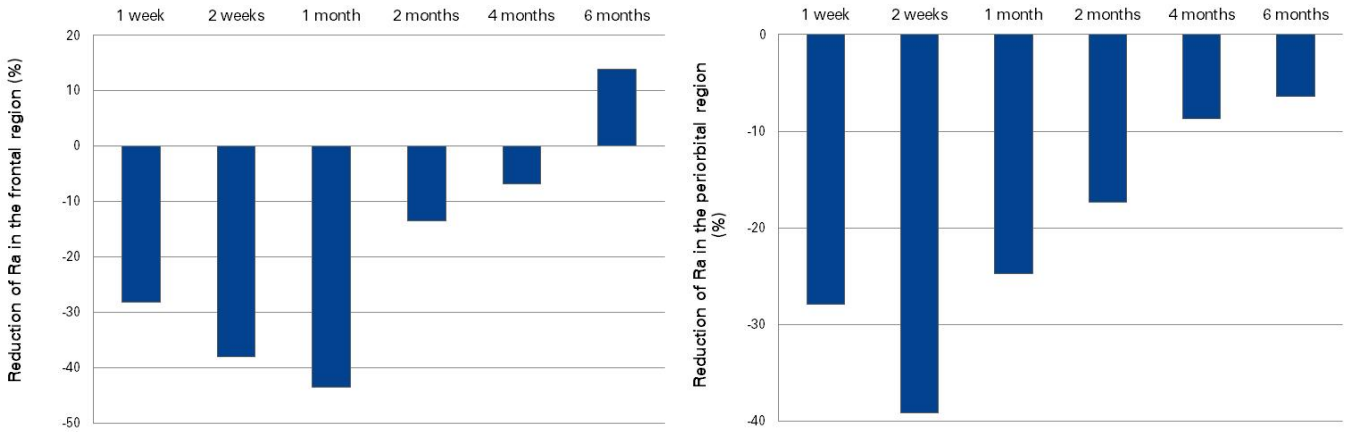

Fig. 2.Skin roughness in the frontal and periorbital regions after the control treatment.

In the frontal region the maximum reduction of skin roughness was observed one month after the injection, drastically diminishing afterwards. Conversely, the maximum reduction in the periorbital region was achieved just two weeks after the injection, gradually decreasing.

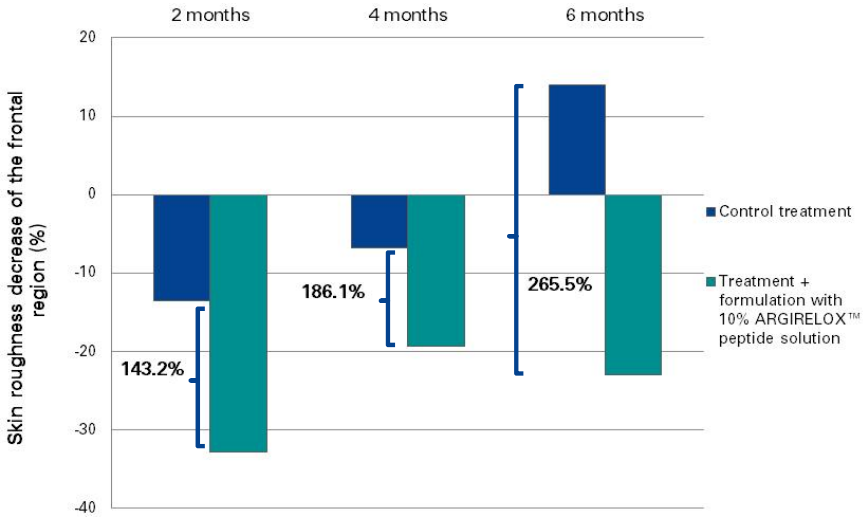

Fig. 3. Skin roughness of the frontal region.

In the **frontal region**, the active topical treatment highly **intensified** the **anti-wrinkle effect** of a BoNT-A injection at all times, prolonging its benefit on the skin even after 6 months.

ARGIRELOX™ *peptide* almost tripled the anti-wrinkle effect of the control treatment in the frontal region.

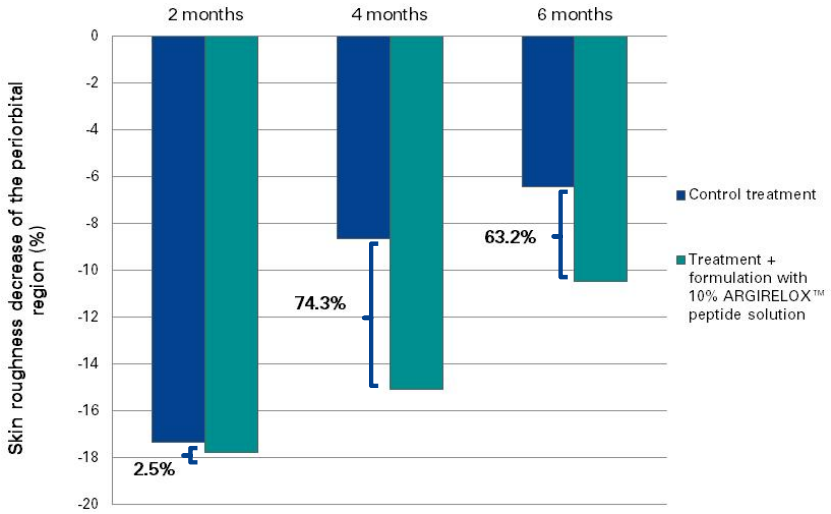

Fig. 4. Skin roughness of the periorbital region.

The active treatment potentiated the smoothing effect on wrinkles induced by the BoNT-A injection in the **periorbital region**, improving the results over **6 months**.

In the crow's feet area, ARGIRELOX™ *peptide* emphasizes the smoothing effect of the control treatment.

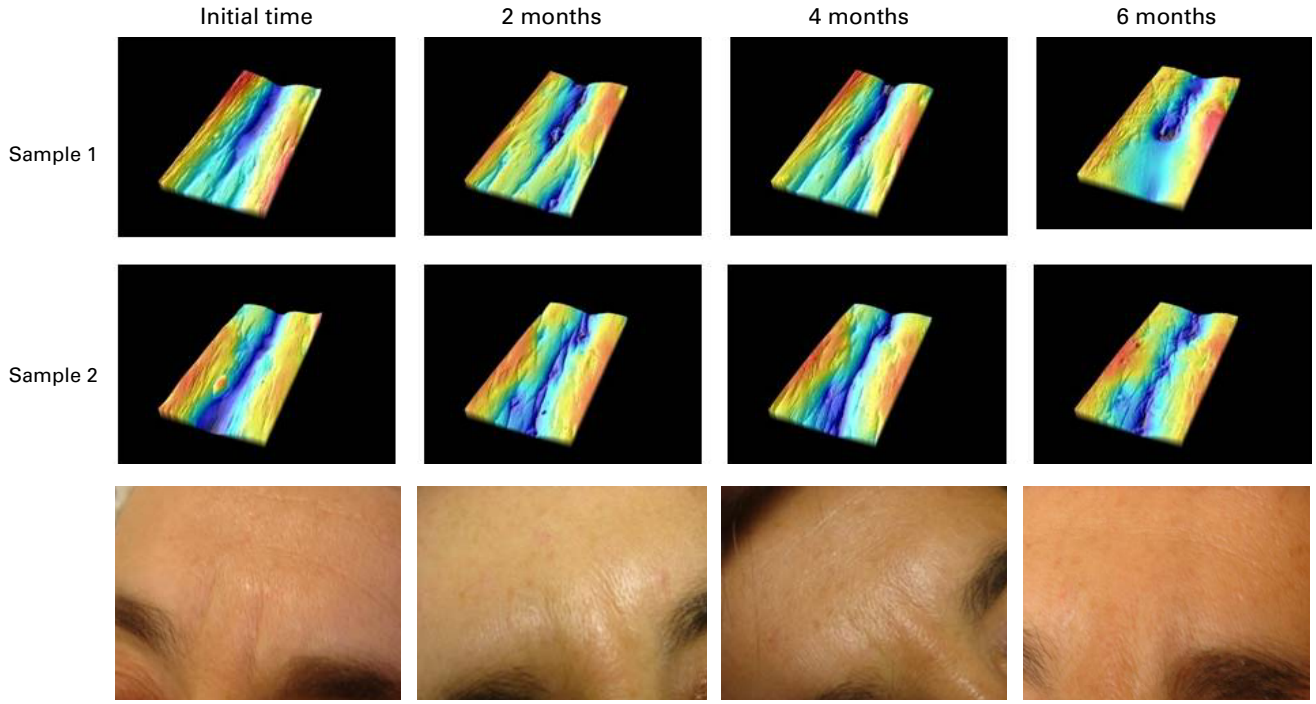

Fig. 5. Images of the frontal region of a volunteer at different times after the toxin injection and active topical treatment.

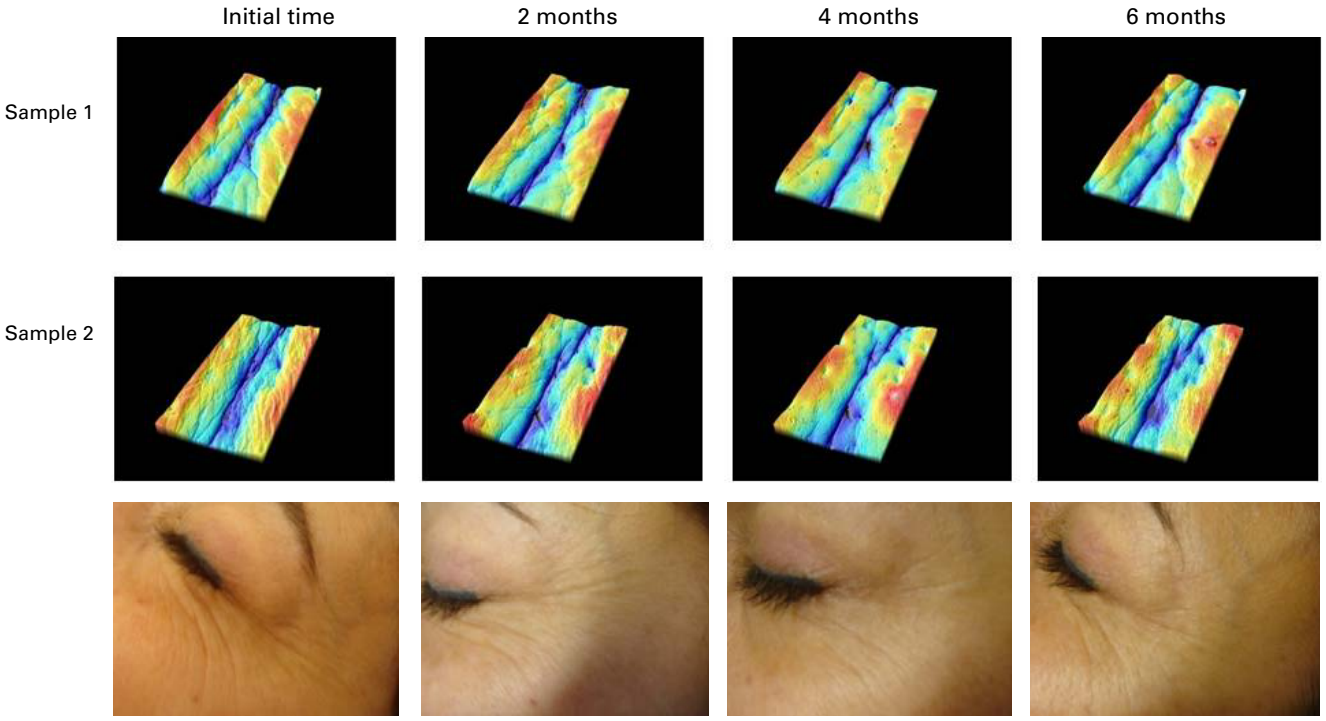

Fig. 6. Real images of the crow's feet area of another volunteer at different times after the BoNT-A injection and active topical treatment.

ARGIRELOX™ *peptide* potentiates the effect of a BoNT-A treatment, extending its anti-wrinkle benefit on the skin.

Cosmetic properties

ARGIRELOX™ *peptide:*

- smoothing effect on expression lines by acting on the SNARE complex and calcium channels, both key factors in their development.

- ingredients **modulate glutamate release**, acting synergistically. The combination of Acetyl Hexapeptide-8 and Pentapeptide-18 induced a -40.0% reduction.

- helps to **maintain** the **anti-wrinkle effect** of a **BoNT-A** injection in the frontal and periorbital region, **prolonging** its effect for 6 months. The benefit of the active treatment (10% ARGIRELOX™ *peptide solution*) was always higher than the control.

- almost **tripled** the **anti-wrinkle effect** of the control treatment in the **frontal region** after 6 months and it **decelerated** the reappearance of **expression lines** in the **crow's feet area**.

Cosmetic applications

ARGIRELOX™ *peptide* is an ideal ingredient to add in **facial formulations designed to reduce expression lines** and wrinkles. Thus, it is perfect to use in topical treatments intended to **complement BoNT-A** injections and prolong their anti-wrinkle effects **between sessions**.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
ARGIRELOX™ *peptide*	Acetyl Hexapeptide-8, Pentapeptide-18

PRESENTATION AND PRESERVATIVE

Solution containing 0.05% Acetyl Hexapeptide-8 and 0.025% Pentapeptide-18.

Code	Product presentation	Preservative
PD260	ARGIRELOX™ *peptide solution*	Preservative free

Application data

PROCESSING

ARGIRELOX™ *peptide* can be formulated in the aqueous phase of emulsions and gels in the final step of the manufacturing process. In case of preparing an emulsion, it should be added once the emulsion is formed. In both cases, it should always be provided that the temperature is below 40 ºC.

Recommended pH range between 3.0 and 8.0 for ARGIRELOX™ *peptide.*

INCOMPATIBILITIES

Oxidants and electrophiles.

SOLUBILITY

Soluble in water.

DOSAGE

A dosage of 10% of ARGIRELOX™ *peptide solution* is recommended in final cosmetic formulations.

References

1. Benedetto AV. Environmental and skin aging. *Clin Derm*. 16:129:139, 1998.

2. Stegman SJ, Tromovitch TA, Clogau RG. The skin of the aging face in cosmetic dermatologic surgery. 2nd ed. Mosby year book. St Louis. MO, pp:5-15, 1990.

3. Blanes-Mira C, Merino JM, Valera E, *et al*. Small peptides patterned after the N-terminus domain of SNAP25 inhibit SNARE complex assembly and regulated exocytosis. *J Neurochem*. 88:124-135, 2004.

4. Chen YA, Scheller RH. SNARE-mediated membrane fusion. Nature Reviews Molecular Cell Biology 2: 98-106, 2001.