Active Beauty

Vetivyne™
Fragrance inspired skin youth booster

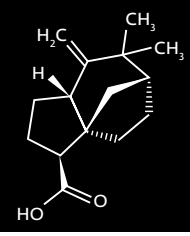
BSB Innovation 1st NATURAL Prize Products 2018 Category Most Innovative Raw Material

Crafted by green technology

Givaudan

engage your senses

Focus on the product



Zizanoic acid and dereplication of Vetiver root extract

Skin lipids and ageing

Skin lipids play a fundamental role in the maintenance of skin hydration, suppleness and barrier function. They are mostly produced by three types of cells:

- Sebocytes produce sebum, a thin lipidic film primarily composed of triglycerides, waxes, esters, squalene, and free fatty acids. Sebum ensures skin protection from dehydration, maintenance of its suppleness and also protects skin microflora by its acidic pH and its Antimicrobial Lipids (AML)[1].
- Keratinocytes accumulate lipids during their differentiation, such as ceramides and cholesterol derivatives, which will further build up the skin cornified envelope of the *stratum corneum*[2]. This process is a key step in the preservation of skin barrier function and also prevents skin dehydration.
- Adipocytes, present in the subcutaneous fat, produce other types of triglycerides for energy storing, mechanical protection, and thermoregulation. They play an important role in skin volume, tonicity and firmness by ensuring mechanical support.

The disruption of the *stratum corneum* integrity usually starts with lipids depletion, with an overall decrease of -30% with age[3]. Lipids produced by the sebocytes are also affected, with a gradual decrease of sebum production around 25% per decade[4]. This results in **a loss of hydration of the skin**.

Meanwhile, facial fat loss upon ageing is related to a lower differentiation of the adipocytes[5], and a decreased fat storage, resulting in **less firmness and tonicity, sagging and wrinkles appearance**.

Upcycling vetiver roots for skin beauty

Vetiver has been a source of inspiration for some of the most iconic fine fragrances in Perfume history. The most exquisite vetiver is grown on the island of Haiti, where Givaudan has been supporting since 2012 a cooperative of vetiver root producers, who have achieved Ecocert Organic Standard and fair trade certification (ESR standard). This initiative ensures the social and environmental responsibility of vetiver production, enabling the use of a fully responsibly-sourced vetiver oil.

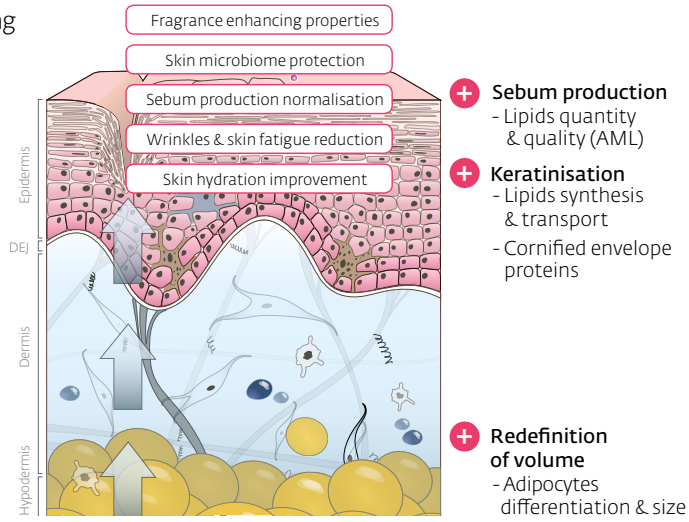
With **state-of-the-art extraction and purification processes**, Active Beauty experts have been able to re-use exhausted vetiver roots to create a fully natural, concentrated and odour-free extract showing impressive skincare benefits: Vetivyne™. Exclusive and patent-pending, rich in zizanoic acid, it has unique properties to reactivate the entire lipids synthesis in the skin.

Vetivyne™: mode of action

Vetivyne™ is a unique upcycled and high performance anti-ageing active, crafted by using a side stream of Vetiver oil extraction. It acts on the three main lipids sources in the skin, by improving the sebum production, the keratinisation and the adipocytes capacity to store fat. Additionally, it increases AML quantity in the sebum, thus promoting a prebiotic-like effect (prebiotics are defined as substances selectively used by host's microorganisms, conferring a health benefit[6]).

Five clinical tests *versus* placebo have highlighted the consumers' benefits of Vetivyne™:

1. **Normalisation of sebum production**
2. Improvement of **skin hydration**
3. **Protection of skin microbiome**
4. **Decrease of skin fatigue** and **reduction of wrinkles**
5. **Boost of fragrance long-lastingness**



[1] Fischer et al. 2014 [2] Feingold et al. 2014 [3] Rogers et al. 1996 [4] Downing et al. 1986 [5] Tchokonia et al. 2010, Coleman et al. 2009, Kirland et al. 1997 [6] ISAPP definition

Biological activity

Increasing lipids production for barrier function (*ex vivo*)

A proteomic analysis was performed by LC-MS/MS on human skin explants of 3 donors (54, 56 and 69 years old women) on which product was applied at 1%, everyday for 6 days.

Cellular function	Proteins name	Fold expression
Lipids metabolism & transport	ORM1 - Alpha 1 acid glyco-protein 1	0.57*
	RTN4 - Reticulon-4	1.33*
	CLTA - Clathrin light chain A	3.77*
	SNX5 - Sorting nexin 5	1.72*
	CERT - Ceramide transport protein	2.69*
	TFG - Protein TFG	1.34**
Epidermis differentiation	INV - Involucrin	1.59
	KRT36 - Keratin type I cuticular Ha6	1.73**
	DMKN - Dermokine	1.53**
	LOR - Loricrin	3.39*

Results: Vetivyne™ **stimulates the expression of a complete set of proteins** involved in the multiple pathways targeting **lipids production, lipids transport, and epidermis differentiation**.

*p<0.05 Student's t-test **p<0.01 Student's t-test

Boosting sebum quantity and quality

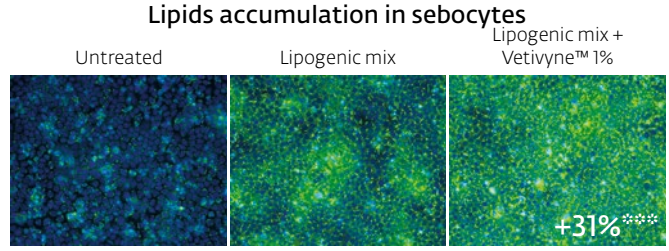
1. Sebocytes stimulation (*in vitro*)

Sebocytes were pre-incubated with or without Vetivyne™ at 1% for 4 hours. Then, a lipogenic mix (vitamin C, vitamin D3, insulin and calcium) was added for 7 days more of incubation. Treatment was renewed after 3 days. The lipid content was then evaluated using Bodipy® fluorescent labeling (lipids were made visible in green).

Results: Vetivyne™ shows a significant **stimulation of sebocytes**, with an increase of **sebum lipids accumulation up to +31%** *versus* lipogenic mix only.

***p<0.001 Student's t test

Lipids accumulation in sebocytes



2. Antimicrobial lipids (AML) content increase (*in vitro*)

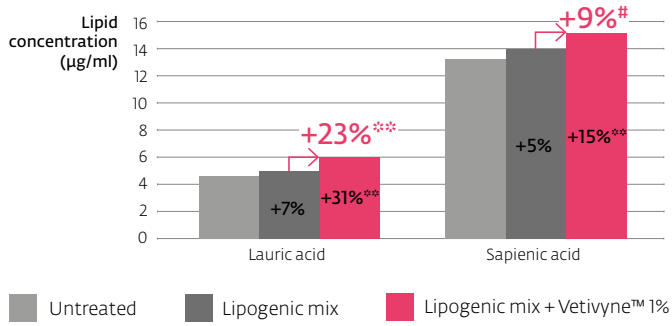
Sebocytes were cultivated in 3D culture and treated in systemic with or without Vetivyne at 1% for 7 days. The content of free fatty acids was then assessed by GC/MS to analyse their content in AML.

Results: Vetivyne™ significantly **increases up to +23% the content of antimicrobial lipids in sebum**.

Vetivyne™ is thus promoting a prebiotic-like effect (prebiotics being defined as substances selectively used by host's microorganisms, conferring a health benefit).

**p<0.01 Student's t-test #p<0.05 Student's t-test

Production of antimicrobial lipids



Biological activity

Barrier function reconstruction

1. Reactivation of lipids production (*in vitro* / *ex vivo*)

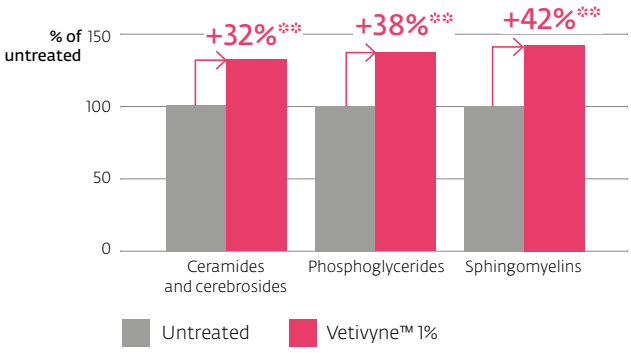
1.1 Ceramides and their precursors synthesis

Reconstructed Human Epidermis (RHE) were incubated with or without Vetivyne™ at 1% for 7 days. Lipids contained in RHE were then extracted and studied by Thin Layer Chromatography and densitometric analysis to assess the quantity of ceramides precusors in the RHE.

Results: Vetivyne™ significantly **increases up to +42% the production of the ceramides and their various precursors in the epidermis.**

**p<0.01 Student's t-test

Ceramides and their precursors synthesis



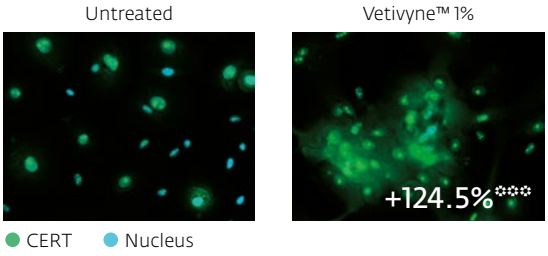
1.2 Ceramides transport stimulation

Normal Human Keratinocytes (NHEKs) were stimulated for 5 days with or without Vetivyne™ at 1%. Ceramide Transport Protein (CERT) was then quantified by immunofluorescence.

Results: Vetivyne™ significantly **increases up to +124.5% the expression of CERT**, enabling a better transport of the lipids in the epidermis.

***p<0.001 Student's t-test

Ceramides transport stimulation



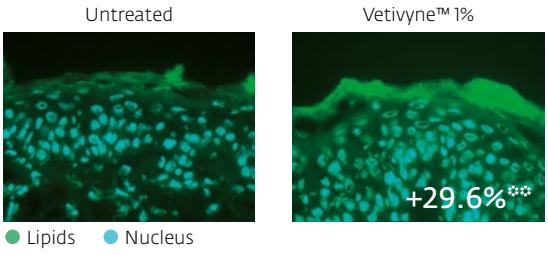
1.3 Boosting lipids content

Skin explants from a donor aged 69 were topically treated with or without Vetivyne™ at 1% for 6 days. The neutral lipids from skin were stained using LipidTOX™. The immunostaining was quantified by microscopical observation (green fluorescence) to assess stimulation of keratinisation.

Results: As a result of boosting lipids production and transport, Vetivyne™ significantly **increases up to +29.6% the lipid content in the cornified envelope**.

**p<0.01 Student's t-test

Boosting lipids content in cornified envelope



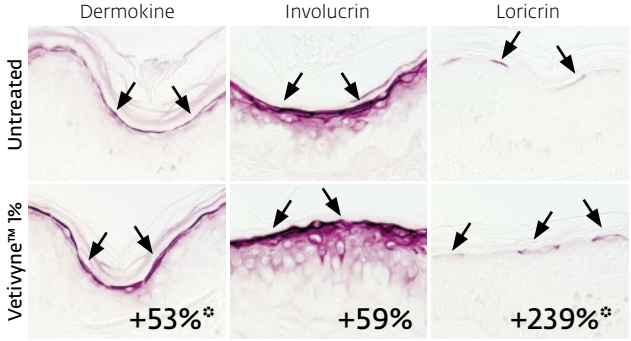
2. Reactivation of skin barrier proteins (*ex vivo*)

Human skin explants from 3 donors (54, 56 and 69 years old women) on which product was applied everyday for 6 days were assessed by proteomic using LC-MS/MS for quantification of dermokine, involucrin and loricrin, 3 characteristic proteins of the cornified envelope.

Results: Vetivyne™ at 1% significantly **increases the expression of 3 major proteins of the *stratum corneum*, from +53% up to +239% in average**.

*p<0.05 Student's t-test

Increase of cornified envelope proteins
(Illustrative pictures from explants of a 69 years old donor)



Biological activity

Boosting skin adipocytes differentiation and size

1. Improving adipocytes differentiation (*in vitro*)

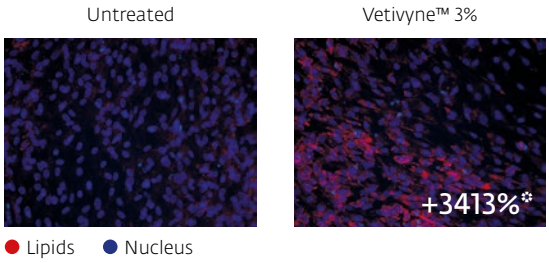
Pre-adipocytes were cultured for 13 days with or without Vetivyne™ at 3%.

Lipid droplets, a marker of adipocytes differentiation, were then labelled using AdipoRed™ (a fluorescent lipid probe) to quantify the differentiation.

Results: Vetivyne™ significantly **increases the adipocytes differentiation, with an effect up to +3413%**.

*p<0.05 Student's t-test

Stimulation of adipocytes differentiation

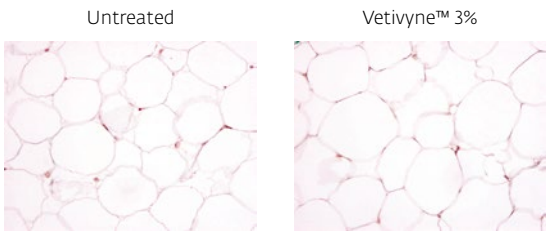


2. Increasing adipocytes volume (*ex vivo*)

Explants of full skin (epidermis, dermis, and hypodermis) were treated or not with Vetivyne™ at 3%. On D0, D1, D4 and D6, the product was topically applied. On D8, explants were stained for microscopical analysis.

Adipocytes size determination was then performed by image analysis, by measuring their equivalent circular diameter.

Increase of adipocytes size



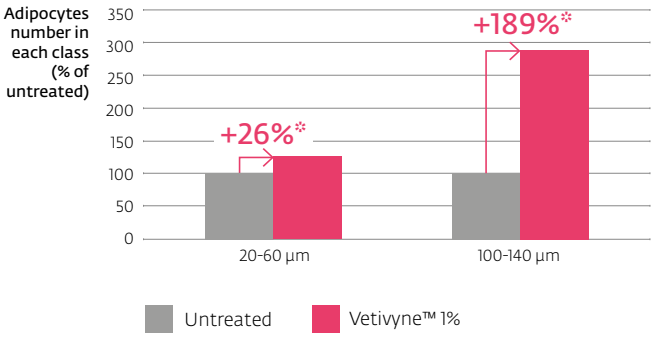
Results: Vetivyne™ significantly **increases the average size of adipocytes in the hypodermis**, as observed by microscopy.

It is also noticeable that a shift in the population of adipocytes can be observed after the use of Vetivyne™:

- The number of « small » adipocytes (20-60 µm) **increases by +26%, reflecting the differentiation boost** observed *in vitro* (appearance of new small adipocytes from pre-adipocytes).
- The number of « large » adipocytes (100-140 µm) **increases by +189%, as a consequence of the average size increase and a better fat storage capacity** (accumulation of lipids in the existing adipocytes).

*p<0.05 Student's t-test

Evolution of adipocytes size distribution and populations



Clinical efficacy

Reactivation of sebum production in mature skin (Clinical test #1)

A double blind clinical evaluation was carried out on 30 volunteers (women from 63 to 70 years old, average of 67), with a low level of sebum on the face.

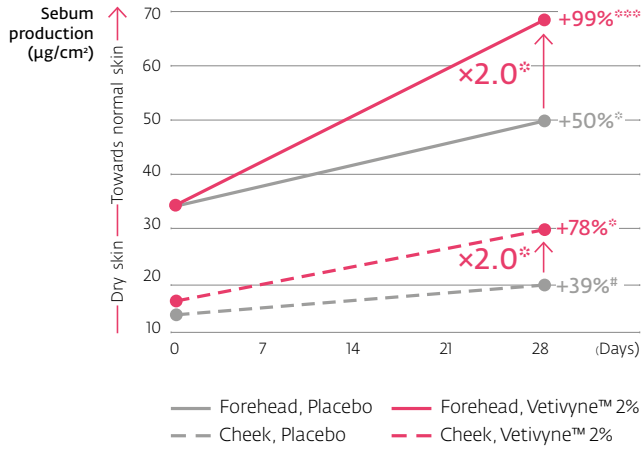
Volunteers applied the product containing Vetivyne™ at 2% or placebo on their face twice a day (morning and evening) for 28 days. At D0 and D28, sebum production was analysed using Sebumeter® on forehead and cheek

Results: Vetivyne™ induces **a significant increase of sebum production** after 28 days of application on both forehead and cheek, 2.0 times more than the placebo.

Sebum production is boosted up to +99% on the forehead, and up to +78% on the cheeks.

Vetivyne™ at 2% is therefore able to reactivate the sebum production, which is usually drastically reduced in mature skin, and to make it closer to normal skin conditions.

Sebum production on cheeks and forehead



***p<0.001 Student's t-test, *p<0.05 Student's t-test, #p<0.1 Student's t-test

Skin organisation and hydration benefits (Clinical test #2)

A double blind clinical evaluation was performed on 20 volunteers (women from 50 to 70 years old, average of 63), with dry legs (corneometry value <35). Volunteers applied the product containing either Vetivyne™ at 2% or a placebo on their legs twice a day (morning and evening) for 28 days.

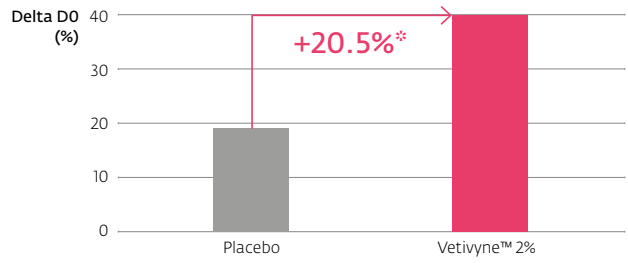
1. Improvement of lipids conformation

At D0 and D28, Raman spectroscopy was used to assess the quality of lipids conformation in the skin.

Results: Vetivyne™ shows a significant **improvement of the lipids conformation in the skin, up to +20.5%,** therefore increasing their compacity, and ultimately the barrier function.

*p<0.05 Student's t-test

Improvement of lipids conformation



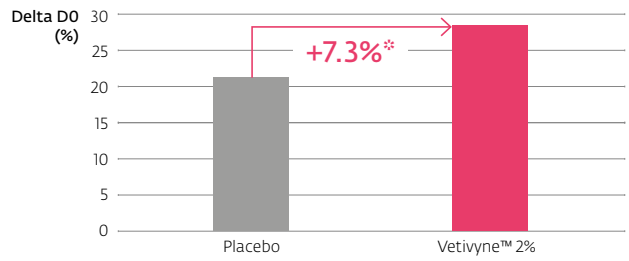
2. Increase of skin hydration

At D0 and D28, skin hydration was analysed by corneometry.

Results: Vetivyne™ shows a significant **increase of skin hydration up to +7.3%** on volunteers with dry skin.

*p<0.05 Student's t-test

Recovery of skin hydration



Clinical efficacy

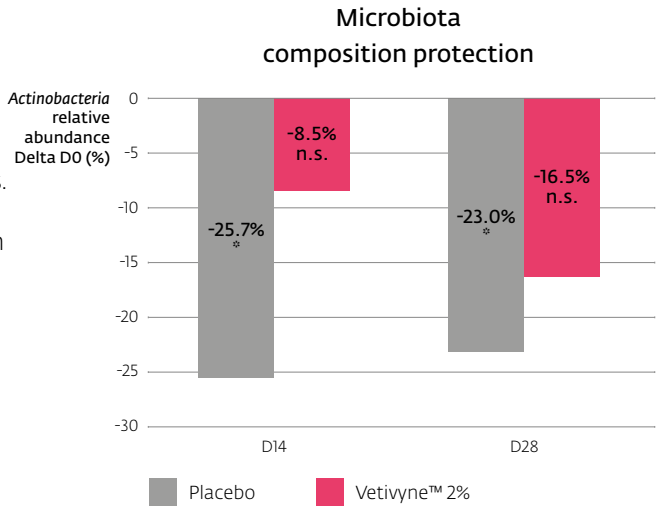
Protection of skin microbiota composition (Clinical test #3)

A microbiome clinical study (16S rRNA) was performed with the same volunteers and conditions than the hydration clinical test (#2). At D0 and D28, volunteers' calves were swabbed, then the 16S rRNA gene (V3V4 region) of the collected microbiomes was extracted and sequenced.
6.2 billions of DNA bases were analysed to compare the evolution of the microflora composition over time under the two conditions.

Results: All of the skin microbiome main phyla were monitored in both conditions. While a significant decrease in the *Actinobacteria* population can be observed at D14 and D28 with the placebo, Vetivyne™ **protects the microbiota composition**, by stabilising it overtime, and avoiding any dysbiosis (no significant evolution of *Actinobacteria*).

No evolutions are observed for the other phyla, either with the placebo or with Vetivyne™.

*p<0.05 Student's t-test, n.s p>0.1 Student's t-test



Visible skin fatigue reduction and decrease of perilabial wrinkles (Clinical test #4)

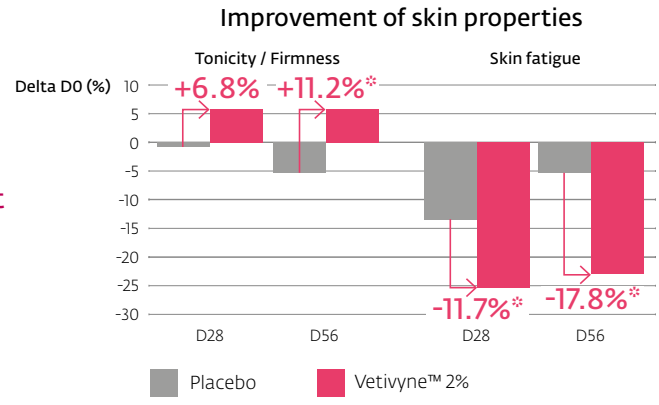
A double blind clinical evaluation was performed on 2 groups of 21 volunteers with sagging face and wrinkles (women from 52 to 69 years old, average of 60). Volunteers applied a placebo or the product containing Vetivyne™ at 2% on their face for 56 days, twice a day (morning and evening).

1. Improvement of tonicity / firmness and anti-fatigue effect

At D0, D28 and D56, skin biomechanical properties were measured by cutometry on cheekbones, focusing on the -R6 and R9 parameters, respectively representative of the viscoelastic balance and of the fatigue effect. At D0, D28 and D56, face of the volunteers was analysed thanks to AEVA HE®, a patented projection unit combined with stereo imaging.

Results: Vetivyne™ enables **a time-progressive and significant recovery of the skin biomechanical properties on aged volunteers**, with **up to +11.2% recovery in tonicity and firmness and -17.8% of skin fatigue decrease** *versus* placebo. These results are clearly visible on the 3D face images of the volunteers.

*p<0.05 Student's t-test



3D face images
(Illustrative pictures from a 58 years old donor)

D0 D28 D56

Clinical efficacy

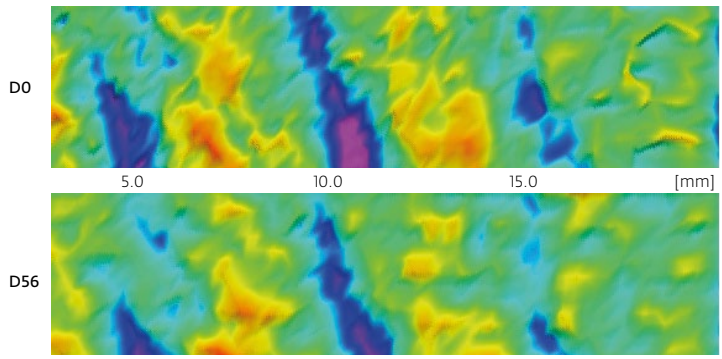
2. Smoothing of perilabial wrinkles

At D0 and D56 a particular focus was put into the perilabial wrinkles of the volunteers to quantify them thanks to AEVA HE®.

Results: The daily application of Vetivyne™ **enables a significant effect for** 100% of the volunteers *versus* placebo in 2 months, with an average **reduction of -18%*** of the perilabial wrinkles**.

***p<0.001 Student's t-test

Decrease of perilabial wrinkles
(Illustrative pictures from a 60 years old donor)



3. Self-assessment of the skin benefits

All volunteers were asked to assess the benefits of Vetivyne™ for their skin at D56.

76%

72%

71%

Fragrance boosting benefits (Clinical test #5)

Thanks to the observed skin benefits (lipids quantity and conformation improvement), hypothesis was made that Vetivyne™ could impact on fragrances behaviour by modifying the skin barrier properties.

To assess the long-lastingness of a fragrance, a clinical test was performed on 20 women. Volunteers applied twice a day a placebo cream on one of their forearm and a cream containing Vetivyne™ at 2% on the other for 1 month. Then, on the last day, a fine fragrance was applied to both of their forearms.

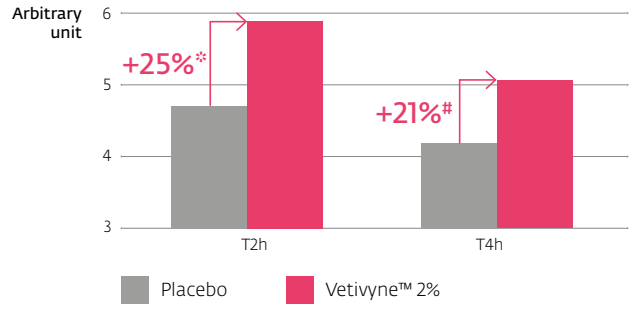
1. Self-assessment of long-lastingness

Volunteers were asked to rank the olfactive intensity of the fine fragrance 2 hours and 4 hours after its application on their skin.

Results: Vetivyne™ demonstrates a **boosting effect of fragrances long-lastingness** by significantly **increasing the fragrance olfactive intensity hours after application, up to +25%**.

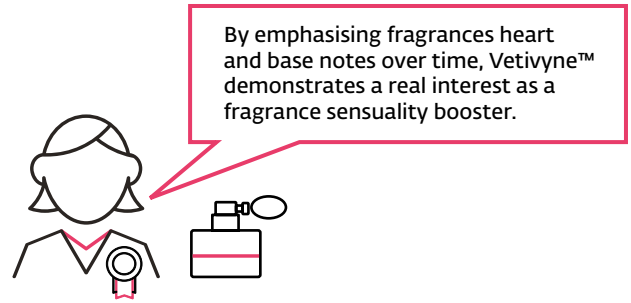
*p<0.05 Student's t-test #p<0.1 Student's t-test

Olfactive intensity (long-lastingness)



2. Professional fragrance evaluator opinion

To confirm the benefits of Vetivyne™ in terms of fragrance boosting properties, a professional fragrance evaluator was asked to assess the differences in terms of olfactive properties between the 2 forearms of the volunteers.



Summary

Technical information

Proposed INCI:	Vetiveria Zizanoides Root Extract (and) Propanediol (and) Water
Origin:	Vegetal extraction
Preservation:	Preservative free
Appearance:	Amber liquid
Solubility:	Water soluble
Dosage:	1-3%
Processing:	Can be added at the beginning or a the end of the formulation process into the water phase. Formulate at temperature below 40°C, and pH between 4 and 10.

Claims

Claims:	Stimulation of sebum production, normalisation of sebum, stimulation of sebum antimicrobial lipids production, protection of skin microbiome, activation of adipocytes volume increase, skin hydration, skin tonicity booster, skin fatigue reduction, perilabial wrinkles reduction, skin repulping, fragrance long-lastingness enhancer.
Applications:	Serums for dry skin, anti ageing perilabial serum, anti -ageing night and day creams, anti fatigue essence, skin tonifying mask, anti-acne cream, body lotion to increase fragrance long-lastingness.

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