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Testing Efficacy

ANALYSIS OF THE ANTI-HAIR LOSS EFFECTS OF MIX CNCE AND SERENOA REPENS IN HUMAN FOLLICLE DERMAL PAPILLA CELLS

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FINAL REPORT

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ANALYSIS OF THE ANTI-HAIR LOSS EFFECTS OF MIX CNCE AND SERENOA REPENS IN HUMAN FOLLICLE DERMAL PAPILLA CELLS

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Executive Resume

GOAL: To determine the *in vitro* effects of MIX CNCE and *Serenoa repens* on *SRD5A1 (5α-Reductase type 1), SRD5A2 (5α-Reductase type 2)* and *SRD5A3 (5α-Reductase type 3)* gene expression, after treatment in Human Follicle Dermal Papilla Cells (HFDPC).

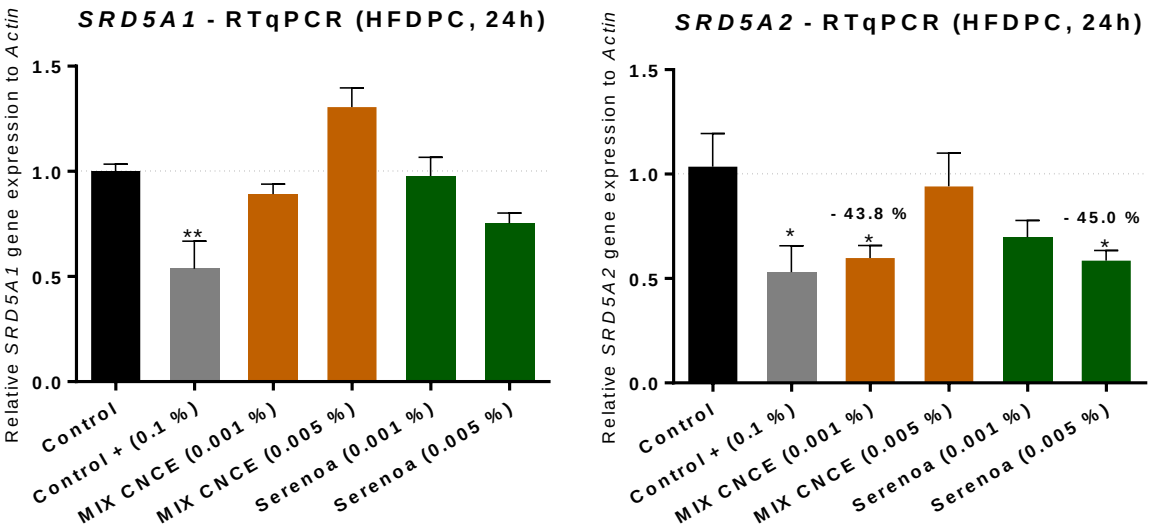
METHODOLOGY: First, the effects of the product on cell viability were determined. Human Follicle Dermal Papilla Cells (HFDPC) were cultured in the presence of different concentrations of MIX CNCE or *Serenoa repens* for 24 hours. After treatment, MTT assay was performed to assess the effects on cell viability, in order to determine cytotoxicity effects and the effective concentrations to be used in subsequent assay. For gene expression analysis, HFDPC cells were treated with MIX CNCE, *Serenoa repens* or positive control for 24 hours. After that, total RNA was purified, quantified and it was used to synthesize complementary DNA (cDNA). This cDNA from treated or untreated cells (control) was used to determine the relative gene expression of *SRD5A1, SRD5A2* and *SRD5A3* through RT-qPCR. *Actin (ACT)* was used as reference gene. Data was statistically analysed.

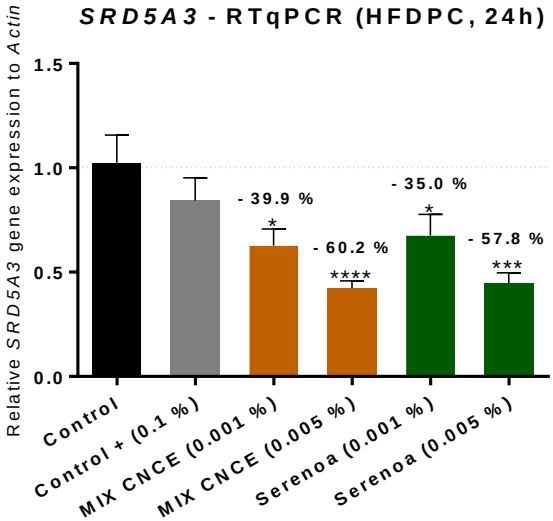
RESULTS: After treatment during 24 hours with MIX CNCE or *Serenoa repens*, cell viability assay indicated that safe working concentrations could start at 0.01 %, since the slight toxicity detected at this or lower concentrations is considered irrelevant. According to results and client's needs, concentrations 0.001 % and 0.005 % were selected for subsequent gene expression study.

Results indicated that treatment with MIX CNCE at 0.001 % concentration significantly inhibited gene expression of SRD5A2 and SRD5A3 by 43.8 ± 13.8 % and 39.9 ± 14.5 %, respectively, compared to the untreated control, whereas the treatment at 0.005 % significantly decreased gene expression of SRD5A3 by 60.2 ± 10.2 %. In parallel, the treatment with *Serenoa repens* at 0.001 % significantly inhibited gene expression of SRD5A3 by 35.0 ± 16.4 %, whereas the treatment at 0.005 % significantly decreased gene expression of SRD5A2 and SRD5A3 by 45.0 ± 12.8 % and 57.8 ± 11.4 %, respectively. No significant results were obtained for both treatments in SRD5A1 gene expression.

CONCLUSION: The ***in vitro*** treatment during 24 hours with **MIX CNCE** or with ***Serenoa repens*** **displays anti-hair loss effects in Human Follicle Dermal Papilla Cells (HFDPC),** through **significant inhibition of *SRD5A2 (5α-Reductase type 2)* and *SRD5A3 (5α-Reductase***

***type 3)* gene expression,** compared to the untreated control. For **SRD5A2**, **MIX CNCE at 0.001 % concentrations shows similar capacity than Serenoa repens at 0.005 %,** whereas for **SRD5A3**, results for **both treatments are in the same range of activity**.





1 Title

Analysis of the anti-hair loss effects of MIX CNCE and *Serenoa repens* in Human Follicle Dermal Papilla Cells.

2 Introduction

Androgenetic alopecia (AGA) or also known as Male-pattern hair loss (MPHL) is the most common type of hair loss, affecting women (50 % of menopausal women and a large number of women of childbearing age, around 25 %), as well as males (over 70 % of adult men) [*McElwee and Saphiro, 2012*]. It occurs due to an underlying susceptibility of hair follicles to shrinkage due to the combined effect of two factors: Genetic predisposition (several loci are involved including AR, EDA2R/Chr. X-WNT10A/2q35, etc.) and hormonal stimulation [*Liang et al., 2013; Rinaldi et al., 2016*]. It is known that both genetic and environmental factors play a role, but many causes of AGA remain unknown.

The hair growth cycle consists of an anagen phase (a growth period of 2-6 years on average), a catagen phase (a period of involution, about 1-3 weeks) and a telogen phase (a rest period of about 1-3 months) [*Geyfman et al., 2014*] as shown in Figure 1. With androgenetic alopecia, under androgenic stimulation, there is a progressive reduction of the average duration of the anagen phase, at each hair growth cycle. The hair follicles become progressively smaller and the hair, shorter and thinner, is absent for longer periods (increased interval between the loss of the hair in telogen and its replacement with new hair), which contributes to worsen the thinned appearance. Hereditary predisposition determines the sensitivity of the follicle to male hormones and thus influences the age of onset and severity of the clinical picture [*Ramos-E-Silva and Pirmez, 2013*].

Androgens control the proliferation of human hair, which responds to hormones differently depending on the body location [*Thornton et al., 1991*]. Dermal papilla cells (DPCs) of the beard, armpit, and scalp hair of people who are genetically predisposed to baldness were shown to be androgen target cells [*Randall, 2007*]. The binding of androgens to their androgen receptors (ARs) decreases the anagen phase of the hair cycle. DPCs have particularly saturable ARs and are proliferated from androgen-responsive follicles. Compared

to testosterone (T), 5α-dihydrotestosterone (DHT) has an approximately five-fold higher affinity for the AR [*Rastegar et al., 2015*].

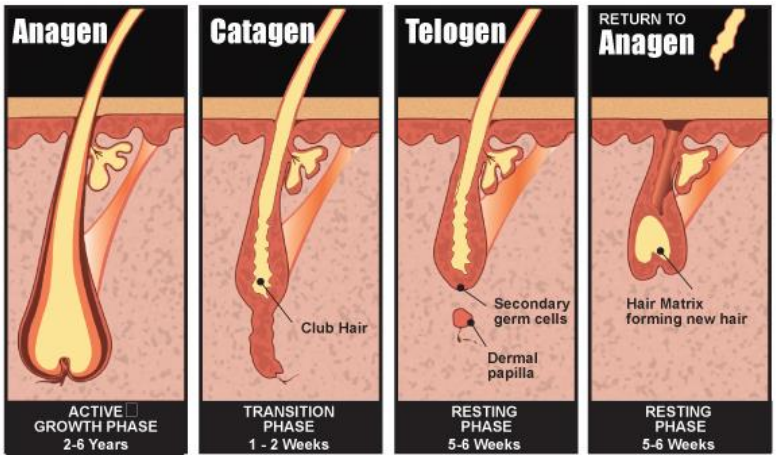


Figure 1. Hair growth phases. *Graphical representation about hair growth phases (Anagen, Catagen and Telogen).*

Enzymes 5α-reductases convert testosterone to 5α-dihydrotestosterone [*Russell and Wilson, 1994*] and this conversion enhance the androgenic signal via two mechanisms: First, DHT cannot be aromatized to estrogen and, therefore, its effect are solely androgenic and in second place, in vitro DHT binds to the AR with a higher affinity than testosterone does, preventing its usual action. A schematic representation of this process is shown in Figure 2. 5α-reductases participate in 3 metabolic pathways: bile acid biosynthesis, androgen and estrogen metabolism, and prostate cancer. It is produced in many tissues in males and females, in the reproductive tract, testes and ovaries, skin, seminal vesicles and pilosebaceous units of hair follicles, among others [*Agís-Balboa et al., 2006*]. There are three isoenzymes of 5α-reductase: steroid 5α-reductase type 1, 2 and 3 (*SRD5A1*, *SRD5A2* and *SRD5A3*) [*Yamana et al., 2010].* Specifically, isoenzymes type 1 and 2 are highly present at pilosebaceuous units in papillae of individual hair follicles [*Bernard, 1994*]. For these reasons, 5α-reductase inhibitors (5-ARIs) have been widely used in the treatment of androgenic alopecia. These agents inhibit the enzyme activity, decreasing conversion of testosterone to DHT, leading to increased testosterone and estradiol; thus preventing hair follicles to shrink and reduce the flow of blood and nutrients.

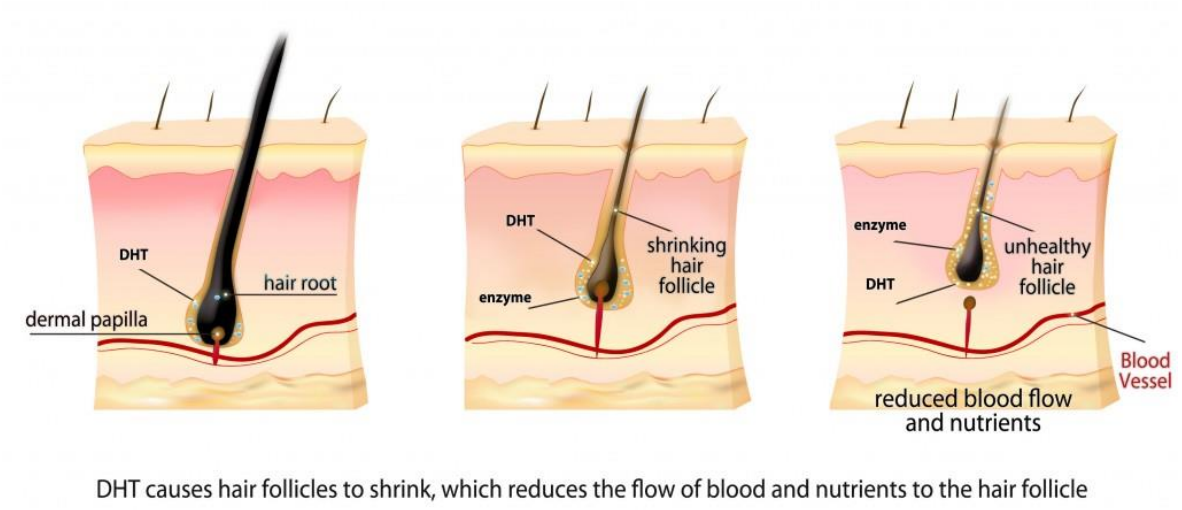


Figure 2. Representation of androgenetic alopecia process. *Schematic representation about the process occurred in androgenetic alopecia, where DHT causes hair follicles to shrink, which reduced the flow of blood and nutrients to the hair follicle, causing alopecia.*

It is widely documented that 5α-reductase enzymes are involved in androgenetic alopecia and that 5α-reductase inhibitors are beneficial treatments for anti-hair loss due to androgenetic alopecia. For these reasons, in this assay, the capacity of MIX CNCE and *Serenoa repens* to inhibit the expression of *SRD5A1 (5α-Reductase type 1), SRD5A2 (5α-Reductase type 2)* and *SRD5A3 (5α-Reductase type 3)* through RT-qPCR, was assessed *in vitro* after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC).

3 Products tested

The following products were received in Bionos on 04/02/2020 at room temperature, and labelled as indicated:

P.1598: MIX CNCE

P.1599: *Serenoa repens*

Samples were stored at room temperature in our facilities to avoid alteration until the start of the experiment and dilutions were freshly prepared each time.

4 Registration date

Study begins: 31/01/2020

Study ends: 02/03/2020

Experimental phase begins: 04/02/2020

Experimental phase ends: 26/02/2020

5 Platform

Human Follicle Dermal Papilla Cells, HFDPC.

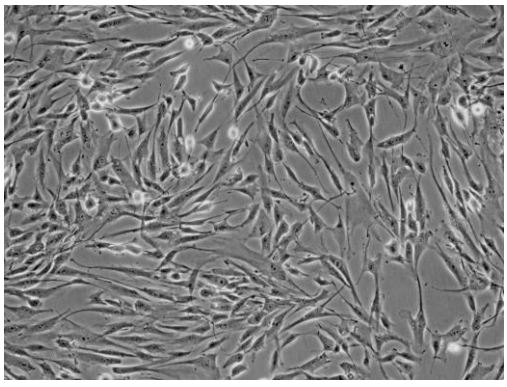


Figure 3. Human Follicle Dermal Papilla Cells. *Microscope image of Human Follicle Dermal Papilla Cells (HFDPC), used during the studies.*

6 Material and methods

6.1 Analytical equipment

Stereoscopic microscope, incubator, refrigerated centrifuge, statistical analysis software, laminar flow hood, Bürker chamber, micropipettes, tips, pipettes, propipette, rack, quantifier Nano-Drop spectrophotometer, Quant studio 5 (Applied Biosystem) Quantitative real-time PCR, vortex, precision balance, heating block and consumables.

6.2 Reagents

HFDPC culture medium (Promocell), nutrient solution mix (Promocell), Phosphate buffered saline (Sigma), Trypan Blue Solution (Bio-Rad), Ethanol (Sigma-Aldrich), MTT reagent (Invitrogen), DMSO (SIGMA), RNAse free-DNAse (Qiagen), PrimeScript RT Reagent (Perfect Real Time- Takara Clontech), Oligonucleotides for RT-PCR amplification of *SRD5A1*, *SRD5A2*, *SRD5A3* and *β-ACT*, SYBR ® qRT-PCR, liquid nitrogen.

6.3 Procedure

For seeding cells, cell numbers and viability were determined using Trypan-Blue staining and counting in a Bürker chamber under the microscope. For the MTT assay, we followed ECVAM Guidelines as established in ECVAM Database Service on Alternative Methods to Animal Experimentation (MTT assay protocol nr. 17). Human Follicle Dermal Papilla Cells (HFDPC) were cultured overnight at a 10.000 cells/well density in a 96-well plate. 24 hours later, cells were treated with different concentrations of MIX CNCE or *Serenoa repens* at 8 different concentrations (3 %, 1 %, 0.3 %, 0.1 %, 0.03 %, 0.01 %, 0.003 % and 0.001 %) diluted in growth medium. After 24 hours of incubation, the medium was removed, and wells were washed with PBS to eliminate any residual medium, and a solution of MTT 1:11 was added to each well. Plates were incubated in the refrigerated incubator at 37ºC for 3 hours. MTT reactive was removed, and DMSO 100% was added to each well to solubilize formazan crystals prior to absorbance measurements at 550 nm and reference of 620 nm.

For the main gene expression assay, HFDPC cells were cultured at a 300.000 cells/well density in a 6-well plate, in growth medium. 24 hours later, the medium was removed and MIX CNCE or *Serenoa repens* at 0.001 % and 0.005 % concentration were added to cells. Positive control at 0.1 % was included in the assay. After 24 hours of incubation period, cells were

washed with PBS buffer and collected in lysis buffer to proceed with RNA extraction. Total RNA was extracted using RNeasy kit (Qiagen) and treated with DNAse-I to remove any contamination from genomic DNA. RNA quality and quantity were checked in a Nano-Drop spectrophotometer, and 500 µg of total RNA was used to synthesize cDNA, using First-strand Synthesis kit (Takara-Clontech). The suitability of each primer pair used in this study for RT-qPCR, *SRD5A1, SRD5A2, SRD5A3* and *ACT* was previously evaluated to determine melting curves, efficiency of amplification and specificity of the primers. Finally, quantitative PCR (qPCR) was performed in a real time PCR machine (QuantStudio 5, Applied BioSystem).

To perform raw data analysis, we used the Pfaffl method [*Pfaffl, 2001*] to calculate the gene relative expression ratio to *ACT* (internal control- housekeeping gene). Mathematical model of relative expression ratio in real-time PCR is shown in Figure 4. Statistical analysis to determine significant changes was performed using Student's t-test. For all data a level of 5% or less ($p < 0.05$) was taken as statistically significant.

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta \text{CP}_{\text{target}}(control - sample)}}{(E_{\text{ref}})^{\Delta \text{CP}_{\text{ref}}(control - sample)}}$$

Figure 4. Mathematical model of relative expression ratio used in real-time PCR data analysis*. The ratio of a target gene is expressed in a sample versus a control in comparison to a reference gene. Etarget is the real-time PCR efficiency of target gene transcript; Eref is the real-time PCR efficiency of a reference gene transcript; ΔCPtarget is the CP deviation control – sample of the target gene transcript; ΔCPref = CP deviation of control – sample of reference gene transcript.*

7 Results

The anti-hair loss capacity of MIX CNCE and *Serenoa repens* was assessed on HFDPC in culture by quantifying the expression of *SRD5A1, SRD5A2* and *SRD5A3* through RT-qPCR. The MTT assay was used to determine non-toxic and working concentrations of MIX CNCE and *Serenoa repens* in cellular cultures.

7.1 Cell viability (MTT)

After MIX CNCE or *Serenoa repens* treatment for 24 hours, cell viability indicated that safe working concentrations could start at 0.01 %, since the slight toxicity detected at this and lower concentrations is considered irrelevant (Figure 5 and Table 1). For *Serenoa repens*, it should be noted that the product at 3 % and 1 % concentration interfered with the OD_{600} readings, showing a false increase in cell viability. To assess the statistical significance of the results we used the ordinary one-way ANOVA test.

According to results and client's needs, concentrations 0.001 % and 0.005 % were selected for subsequent gene expression study.

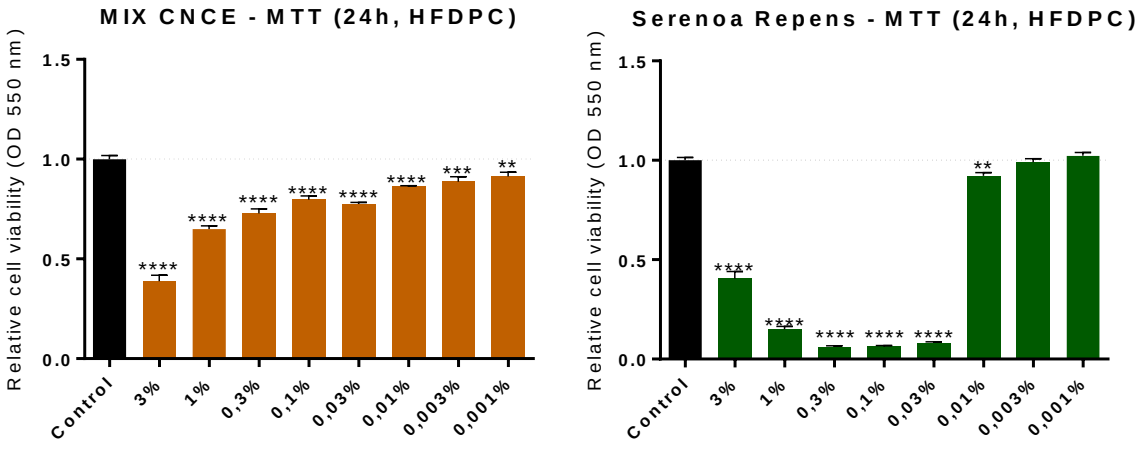


Figure 5. Cell viability results. *Graphical representation of the results showing cell viability after treatment for 24 hours with MIX CNCE and Serenoa Repens at 3 %, 1 %, 0.3 %, 0.1 %, 0.03 %, 0.01 %, 0.003 % and 0.001 %. ** Represents statistical significance with p value < 0.01. *** Represents statistical significance with p-value < 0.001. **** Represents statistical significance with p value < 0.0001.*

MIX CNCE - MTT								
Dunnett's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant?**	**Summary**				
Control vs. 3%	0,6119	0,5415 to 0,6822	**Yes**	****				
Control vs. 1%	0,3510	0,2807 to 0,4213	**Yes**	****				
Control vs. 0,3%	0,2715	0,2012 to 0,3418	**Yes**	****				
Control vs. 0,1%	0,2030	0,1327 to 0,2733	**Yes**	****				
Control vs. 0,03%	0,2245	0,1542 to 0,2948	**Yes**	****				
Control vs. 0,01%	0,1379	0,06753 to 0,2082	**Yes**	****				
Control vs. 0,003%	0,1135	0,04315 to 0,1838	**Yes**	***				
Control vs. 0,001%	0,08625	0,01590 to 0,1566	**Yes**	**				
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**	**n2**	**q**	**DF**
Control vs. 3%	0,9999	0,3880	**0,6119**	0,02586	16	8	23,66	71
Control vs. 1%	0,9999	0,6489	**0,3510**	0,02586	16	8	13,57	71
Control vs. 0,3%	0,9999	0,7284	**0,2715**	0,02586	16	8	10,50	71
Control vs. 0,1%	0,9999	0,7969	**0,2030**	0,02586	16	8	7,849	71
Control vs. 0,03%	0,9999	0,7754	**0,2245**	0,02586	16	8	8,680	71
Control vs. 0,01%	0,9999	0,8620	**0,1379**	0,02586	16	8	5,331	71
Control vs. 0,003%	0,9999	0,8864	**0,1135**	0,02586	16	8	4,389	71
Control vs. 0,001%	0,9999	0,9136	**0,08625**	0,02586	16	8	3,335	71

***Serenoa repens* - MTT**								
Dunnett's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant?**	**Summary**				
Control vs. 3%	0,5961	0,5348 to 0,6575	**Yes**	****				
Control vs. 1%	0,8510	0,7897 to 0,9123	**Yes**	****				
Control vs. 0,3%	0,9380	0,8767 to 0,9993	**Yes**	****				
Control vs. 0,1%	0,9365	0,8752 to 0,9978	**Yes**	****				
Control vs. 0,03%	0,9185	0,8572 to 0,9798	**Yes**	****				
Control vs. 0,01%	0,07850	0,01715 to 0,1398	**Yes**	**				
Control vs. 0,003%	0,009625	-0,05172 to 0,07097	No	ns				
Control vs. 0,001%	-0,02325	-0,08460 to 0,03810	No	ns				
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**	**n2**	**q**	**DF**
Control vs. 3%	1,000	0,4039	**0,5961**	0,02255	16	8	26,43	71
Control vs. 1%	1,000	0,1490	**0,8510**	0,02255	16	8	37,73	71
Control vs. 0,3%	1,000	0,0620	**0,9380**	0,02255	16	8	41,59	71
Control vs. 0,1%	1,000	0,0635	**0,9365**	0,02255	16	8	41,52	71
Control vs. 0,03%	1,000	0,0815	**0,9185**	0,02255	16	8	40,72	71
Control vs. 0,01%	1,000	0,9215	**0,07850**	0,02255	16	8	3,480	71
Control vs. 0,003%	1,000	0,9904	0,009625	0,02255	16	8	0,4267	71
Control vs. 0,001%	1,000	1,023	-0,02325	0,02255	16	8	1,031	71

Table 1. *Statistical analysis of the results shown in Figure 5.*

7.2 Primer pair validation

To evaluate the correct amplification and specificity of primer pairs for the genes of interest, melting curves for each primer pair were performed. Results showed an efficient amplification of the genes, with a single peak in the melting point, indicating the high specificity of the primers and their suitability for real-time application, as they do not form primer-dimer structures. The melting curves for each primer pair used in the assay are shown below. As shown, all the oligos amplify one amplicon and the melting temperatures are in the same range.

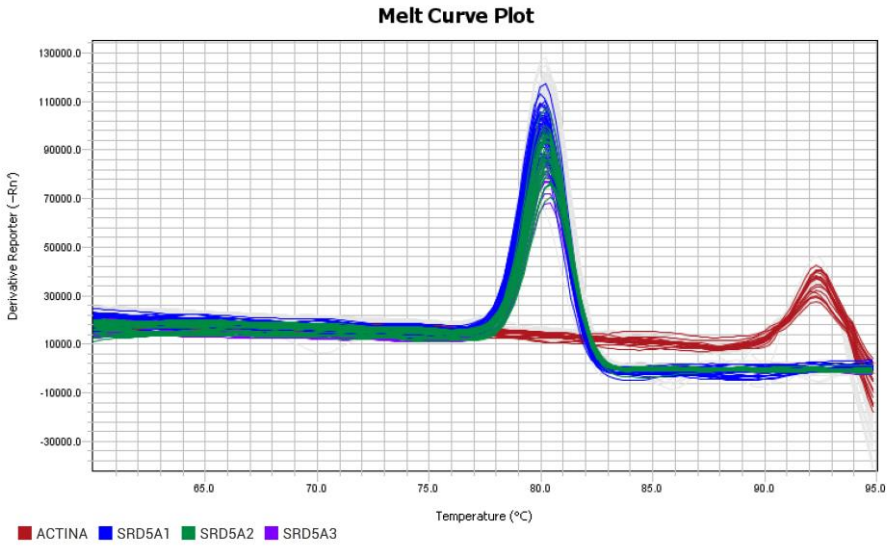


Figure 6. Melt Curve Plot. *Melting curves showing a single peak for all the oligos used in this qPCR assay.*

In Figure 7, it is shown a plot of the technical parameters taken into account for the analysis of the data. All together the parameters indicate that the efficiency of the PCR reaction was optimal.

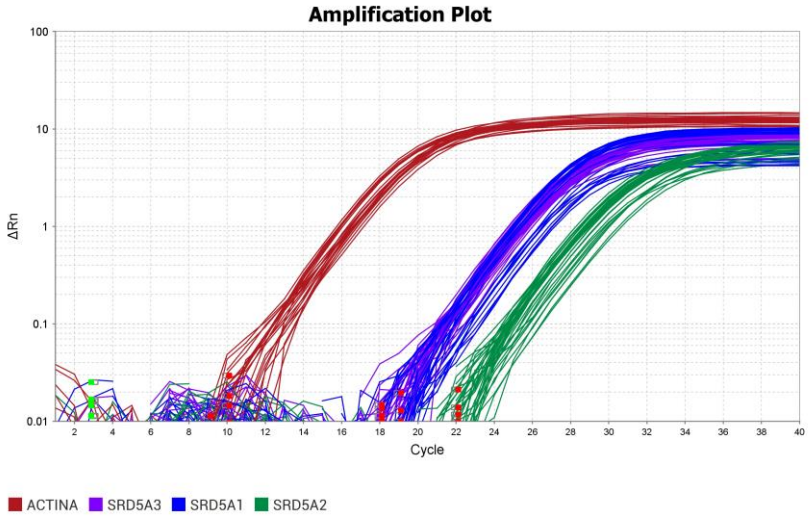


Figure 7. Parameters evaluated to check technical quality of the RT-qPCR reaction. *Amplification plot vs cycle represent the magnitude of normalized fluorescence signal generated by the reporter at each cycle during PCR amplification; in plot amplification Ct vs Well, Ct indicates the PCR cycle number at which the fluorescence meets the threshold in the amplification plot; good Ct values should stay in the range >8-35<; the plot Fluorescence vs Cycle indicates the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference. All these parameters allow identifying and examining irregularity in the amplification.*

7.3 Gene expression quantification by qPCR

mRNA expression levels were determined after treatment with the product tested in Human Follicle Dermal Papilla Cells (HFDPC) for 24 hours. *SRD5A1, SRD5A2, SRD5A3* and *ACT* (internal control) were amplified using four technical replicates of cDNAs.

Results indicated that treatment with MIX CNCE at 0.001 % concentration significantly inhibited gene expression of SRD5A2 and SRD5A3 by 43.8 ± 13.8 % and 39.9 ± 14.5 %, respectively, compared to the untreated control, whereas the treatment at 0.005 % significantly decreased gene expression of SRD5A3 by 60.2 ± 10.2 %.

In parallel, the treatment with *Serenoa repens* at 0.001 % significantly inhibited gene expression of SRD5A3 by 35.0 ± 16.4 %, whereas the treatment at 0.005 % significantly decreased gene expression of SRD5A2 and SRD5A3 by 45.0 ± 12.8 % and 57.8 ± 11.4 %, respectively.

No significant results were obtained for both treatments in SRD5A1 gene expression; as shown in Figure 8 and Table 2.

The treatment with positive control at 0.1 % significantly decreased *SRD5A1* and *SRD5A2* gene expression by 46.5 ± 15.1 % and 50.5 ± 20.1 %, respectively, compared to the untreated control

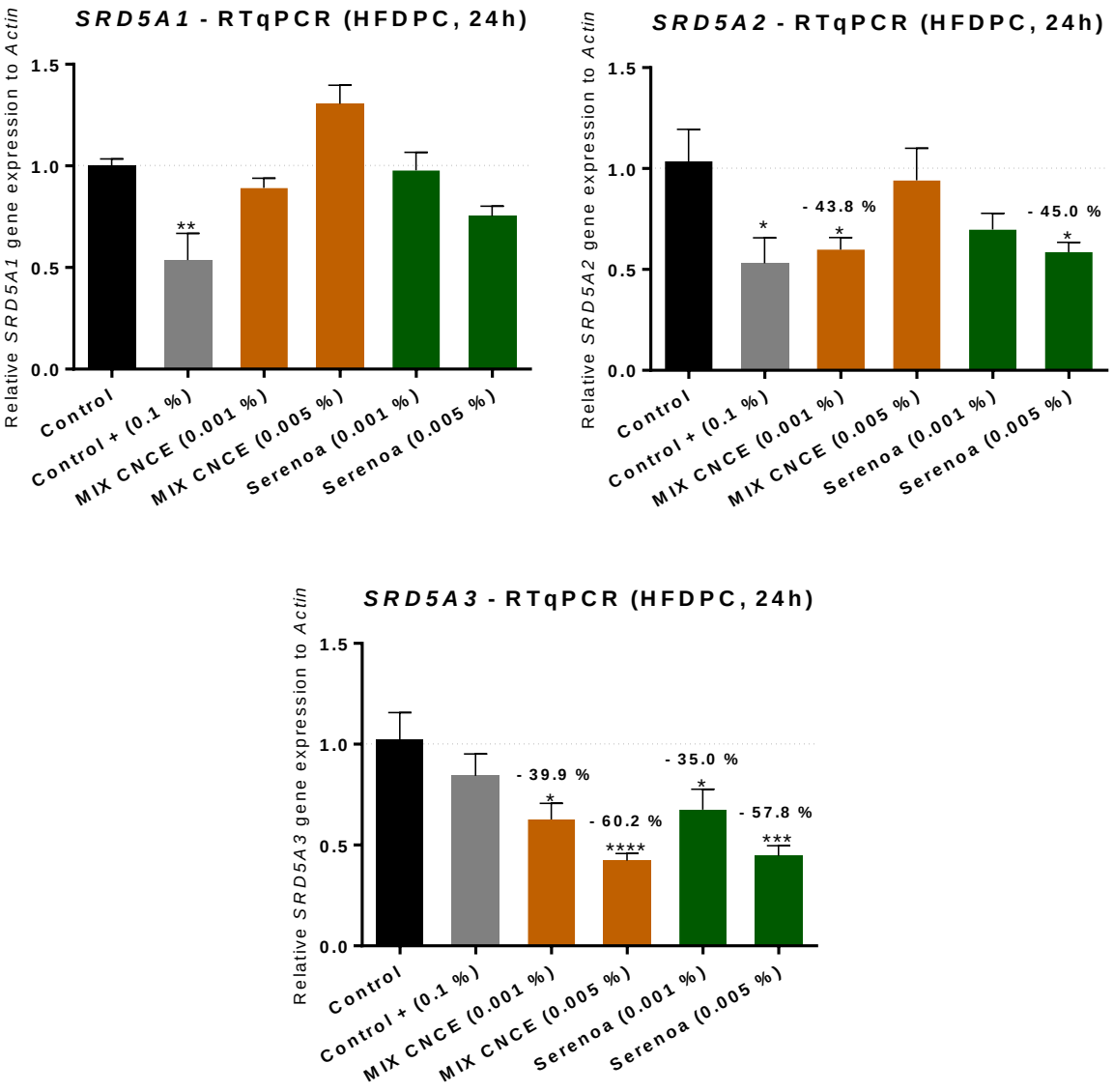


Figure 8. Gene expression results. *Bar graphs showing SRD5A1, SRD5A2 and SRD5A3 gene expression results after treating HFDPC cells with MIX CNCE or Serenoa repens at 0.001 % and 0.005 % concentrations or with positive control at 0.1 % concentration during 24 hours, compared to untreated Control. * Represents statistical significance with p value < 0.05. ** Represents statistical significance with p value < 0.01. *** Represents statistical significance with p-value < 0.001. **** Represents statistical significance with p value < 0.0001.*

***SRD5A1* – RT-qPCR**								
Dunnett's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant?**	**Summary**				
Control vs. Control + (0.1 %)	0,4648	0,1296 to 0,7999	**Yes**	**				
Control vs. MIX CNCE (0.001 %)	0,1095	-0,1965 to 0,4155	No	ns				
Control vs. MIX CNCE (0.005 %)	-0,3041	-0,6266 to 0,01842	No	ns				
Control vs. Serenoa (0.001 %)	0,02425	-0,2983 to 0,3468	No	ns				
Control vs. Serenoa (0.005 %)	0,2483	-0,05770 to 0,5542	No	ns				
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**	**n2**	**q**	**DF**
Control vs. Control + (0.1 %)	1,002	0,537	**0,4648**	0,1264	4	5	3,678	31
Control vs. MIX CNCE (0.001 %)	1,002	0,8923	0,1095	0,1154	4	8	0,9492	31
Control vs. MIX CNCE (0.005 %)	1,002	1,306	-0,3041	0,1216	4	6	2,501	31
Control vs. Serenoa (0.001 %)	1,002	0,9775	0,02425	0,1216	4	6	0,1994	31
Control vs. Serenoa (0.005 %)	1,002	0,7535	0,2483	0,1154	4	8	2,152	31

***SRD5A2* – RT-qPCR**								
Dunnett's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant?**	**Summary**				
Control vs. Control + (0.1 %)	0,5054	0,06291 to 0,9479	**Yes**	*				
Control vs. MIX CNCE (0.001 %)	0,4380	0,03406 to 0,8419	**Yes**	*				
Control vs. MIX CNCE (0.005 %)	0,09400	-0,3318 to 0,5198	No	ns				
Control vs. Serenoa (0.001 %)	0,3376	-0,1049 to 0,7801	No	ns				
Control vs. Serenoa (0.005 %)	0,4503	0,04631 to 0,8542	**Yes**	*				
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**	**n2**	**q**	**DF**
Control vs. Control + (0.1 %)	1,036	0,5306	**0,5054**	0,1665	4	5	3,035	30
Control vs. MIX CNCE (0.001 %)	1,036	0,5980	**0,4380**	0,1520	4	8	2,881	30
Control vs. MIX CNCE (0.005 %)	1,036	0,9420	0,09400	0,1603	4	6	0,5866	30
Control vs. Serenoa (0.001 %)	1,036	0,6984	0,3376	0,1665	4	5	2,027	30
Control vs. Serenoa (0.005 %)	1,036	0,5858	**0,4503**	0,1520	4	8	2,962	30

***SRD5A3* – RT-qPCR**								
Dunnett's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant?**	**Summary**				
Control vs. Control + (0.1 %)	0,1814	-0,1616 to 0,5244	No	ns				
Control vs. MIX CNCE (0.001 %)	0,3985	0,06844 to 0,7286	**Yes**	*				
Control vs. MIX CNCE (0.005 %)	0,6015	0,2884 to 0,9146	**Yes**	****				
Control vs. Serenoa (0.001 %)	0,3498	0,01978 to 0,6799	**Yes**	*				
Control vs. Serenoa (0.005 %)	0,5778	0,2646 to 0,8909	**Yes**	***				
Test details	**Mean 1**	**Mean 2**	**Mean Diff,**	**SE of diff,**	**n1**	**n2**	**q**	**DF**
Control vs. Control + (0.1 %)	1,025	0,8436	0,1814	0,1293	4	5	1,403	31
Control vs. MIX CNCE (0.001 %)	1,025	0,6265	**0,3985**	0,1245	4	6	3,202	31
Control vs. MIX CNCE (0.005 %)	1,025	0,4235	**0,6015**	0,1181	4	8	5,095	31
Control vs. Serenoa (0.001 %)	1,025	0,6752	**0,3498**	0,1245	4	6	2,811	31
Control vs. Serenoa (0.005 %)	1,025	0,4473	**0,5778**	0,1181	4	8	4,893	31

Table Analyzed	***SRD5A1***	***SRD5A1***	***SRD5A1***	***SRD5A1***	***SRD5A1***
Column B	Control + (0,1 %)	MIX CNCE (0,001 %)	MIX CNCE (0,005 %)	Serenoa (0,001 %)	Serenoa (0,005 %)
vs.	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control
Unpaired t test					
P value	0,0181	0,1571	0,0317	0,8379	0,0067
P value summary	*	ns	*	ns	**
Significantly different? (P < 0.05)	**Yes**	No	**Yes**	No	**Yes**
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=3,069 df=7	t=1,530 df=10	t=2,599 df=8	t=0,2114 df=8	t=3,404 df=10
How big is the difference?					
Mean ± SEM of column A	1,002 ± 0,03247 N=4	1,002 ± 0,03247 N=4	1,002 ± 0,03247 N=4	1,002 ± 0,03247 N=4	1,002 ± 0,03247 N=4
Mean ± SEM of column B	0,5370 ± 0,1312 N=5	0,8923 ± 0,04706 N=8	1,306 ± 0,09133 N=6	0,9775 ± 0,08945 N=6	0,7535 ± 0,04803 N=8
Difference between means	**-0,4648 ± 0,1514**	-0,1095 ± 0,07159	**0,3041 ± 0,1170**	-0,02425 ± 0,1147	**-0,2483 ± 0,07293**
95% confidence interval	-0,8228 to -0,1067	-0,2690 to 0,05001	0,03425 to 0,5739	-0,2888 to 0,2403	-0,4108 to -0,08575
R square	0,5737	0,1896	0,4577	0,005554	0,5367

Table Analyzed	***SRD5A2***	***SRD5A2***	***SRD5A2***	***SRD5A2***	***SRD5A2***
Column B	Control + (0,1 %)	MIX CNCE (0,001 %)	MIX CNCE (0,005 %)	Serenoa (0,001 %)	Serenoa (0,005 %)
vs.	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control
Unpaired t test					
P value	0,0397	0,0098	0,6999	0,0822	0,0056
P value summary	*	**	ns	ns	**
Significantly different? (P < 0.05)	**Yes**	**Yes**	No	No	**Yes**
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=2,521 df=7	t=3,183 df=10	t=0,3995 df=8	t=2,027 df=7	t=3,514 df=10
How big is the difference?					
Mean ± SEM of column A	1,036 ± 0,1589 N=4	1,036 ± 0,1589 N=4	1,036 ± 0,1589 N=4	1,036 ± 0,1589 N=4	1,036 ± 0,1589 N=4
Mean ± SEM of column B	0,5306 ± 0,1269 N=5	0,5980 ± 0,06000 N=8	0,9420 ± 0,1591 N=6	0,6984 ± 0,08004 N=5	0,5858 ± 0,04903 N=8
Difference between means	**-0,5054 ± 0,2005**	**-0,4380 ± 0,1376**	-0,09400 ± 0,2353	-0,3376 ± 0,1665	**-0,4503 ± 0,1281**
95% confidence interval	-0,9794 to -0,03136	-0,7446 to -0,1314	-0,6365 to 0,4485	-0,7313 to 0,05615	-0,7357 to -0,1648
R square	0,4759	0,5033	0,01956	0,3700	0,5525

Table Analyzed	***SRD5A3***	***SRD5A3***	***SRD5A3***	***SRD5A3***	***SRD5A3***
Column B	Control + (0,1 %)	MIX CNCE (0,001 %)	MIX CNCE (0,005 %)	Serenoa (0,001 %)	Serenoa (0,005 %)
vs.	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control
Unpaired t test					
P value	0,3191	0,0252	0,0001	0,0654	0,0005
P value summary	ns	*	***	ns	***
Significantly different? (P < 0.05)	No	**Yes**	**Yes**	No	**Yes**
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=1,072 df=7	t=2,746 df=8	t=5,916 df=10	t=2,134 df=8	t=5,075 df=10
How big is the difference?					
Mean ± SEM of column A	1,025 ± 0,1318 N=4	1,025 ± 0,1318 N=4	1,025 ± 0,1318 N=4	1,025 ± 0,1318 N=4	1,025 ± 0,1318 N=4
Mean ± SEM of column B	0,8436 ± 0,1088 N=5	0,6265 ± 0,08078 N=6	0,4235 ± 0,03461 N=8	0,6752 ± 0,1012 N=6	0,4473 ± 0,04946 N=8
Difference between means	-0,1814 ± 0,1692	**-0,3985 ± 0,1451**	**-0,6015 ± 0,1017**	-0,3498 ± 0,1639	**-0,5778 ± 0,1138**
95% confidence interval	-0,5814 to 0,2186	-0,7331 to -0,06385	-0,8280 to -0,3750	-0,7279 to 0,02819	-0,8314 to -0,3241
R square	0,1411	0,4852	0,7778	0,3628	0,7203

Table 2. *Statistical analysis of the results shown in Figure 8.*

8 Discussion and Conclusions

Androgenetic alopecia (AGA) or also known as Male-pattern hair loss (MPHL) is the most common type of hair loss, affecting women (50 % of menopausal women and a large number of women of childbearing age, around 25 %), as well as males (over 70 % of adult men). It occurs due to an underlying susceptibility of hair follicles to shrinkage due to the combined effect of two factors: Genetic predisposition (several loci are involved including AR, EDA2R/Chr. X-WNT10A/2q35, etc.) and hormonal stimulation. It is known that both genetic and environmental factors play a role, but many causes of AGA remain unknown.

Enzymes 5α-reductases convert testosterone to 5α-dihydrotestosterone and this conversion enhance the androgenic signal via two mechanisms: First, DHT cannot be aromatized to estrogen and, therefore, its effect are solely androgenic and in second place, in vitro DHT binds to the AR with a higher affinity than testosterone does, preventing its usual action. There are three isoenzymes of 5α-reductase: steroid 5α-reductase type 1, 2 and 3 (*SRD5A1*, *SRD5A2* and *SRD5A3*).

It is widely documented that 5α-reductase enzymes are involved in androgenetic alopecia and that 5α-reductase inhibitors are beneficial treatments for anti-hair loss due to androgenetic alopecia. For these reasons, in this assay, the capacity of MIX CNCE and *Serenoa repens* to inhibit the expression of *SRD5A1 (5α-Reductase type 1), SRD5A2 (5α-Reductase type 2)* and *SRD5A3 (5α-Reductase type 3)* through RT-qPCR, was assessed *in vitro* after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC).

After treatment during 24 hours with MIX CNCE or *Serenoa repens*, cell viability assay indicated that **safe working concentrations could start at 0.01 %**, since the slight toxicity detected at this or lower concentrations is considered irrelevant. According to results and client's needs, **concentrations 0.001 % and 0.005 % were selected for subsequent gene expression study**.

Results indicated that treatment with **MIX CNCE at 0.001 % concentration significantly inhibited gene expression of SRD5A2 and SRD5A3 by 43.8 ± 13.8 % and 39.9 ± 14.5 %**, respectively, compared to the untreated control, whereas the **treatment at 0.005 % significantly decreased gene expression of SRD5A3 by 60.2 ± 10.2 %**. In parallel, the treatment with ***Serenoa repens* at 0.001 % significantly inhibited gene expression of SRD5A3**

by 35.0 ± 16.4 %, whereas the **treatment at 0.005 % significantly decreased gene expression of SRD5A2 and SRD5A3 by 45.0 ± 12.8 % and 57.8 ± 11.4 %,** respectively. No significant results were obtained for both treatments in SRD5A1 gene expression.

In conclusion, the ***in vitro*** treatment during 24 hours with **MIX CNCE** or with ***Serenoa repens*** **displays anti-hair loss effects in Human Follicle Dermal Papilla Cells (HFDPC),** through **significant inhibition of *SRD5A2 (5α-Reductase type 2)* and *SRD5A3 (5α-Reductase type 3)* gene expression,** compared to the untreated control. For **SRD5A2**, **MIX CNCE at 0.001 % concentrations shows similar capacity than Serenoa repens at 0.005 %,** whereas for **SRD5A3**, results for **both treatments are in the same range of activity**.

9 References

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10 Registry and Regulation

The final report, the raw data and the assay protocol have been saved in computer format, and a copy on paper. All the information provided from the Client, volunteers and generated by Bionos Biotech will be considered as *confidential*. The information about materials, reagents and protocols adopted by Bionos Biotech SL during the assays is confidential and will not be shared with third parts.

The whole process involving this assay was performed under **Quality Management System UNE-EN-ISO 9001/2015.**

Based on **Article 20 or Regulation (EC) No 1223/2009** on cosmetic products (CPR), **Commission Regulation (EU) No 655/2013** established EU harmonised common criteria in order to assess whether or not the use of a claim is justified.

Experimental studies include (but are not limited to) studies ***in silico***, ***in vitro***, ***ex-vivo***, with instrumental or biochemical methods, studies conducted on volunteers, investigator evaluations, sensory evaluations, etc. Different types of experimental studies can be used to provide data on the performance of cosmetic products. It is useful to take into consideration existing relevant guidelines, e.g. guidelines relating to instrumental clinical techniques, other European or international guidelines or standards (e.g. CEN, ISO, etc.).

Such studies should comprise methods which are **reliable and reproducible**. The studies should follow a well-designed and **scientifically valid methodology** according to best practices. The criteria used for evaluation of product performance should be defined with accuracy and chosen in accordance with the aim of the test. The experimental aspect of studies calls for reliance on knowledge and awareness of statistical principles in the design and analysis of the study, e.g. in terms of number of subjects, test samples, etc. This is necessary in order to ensure that the studies achieve scientifically and statistically valid conclusions.

A study protocol should be drawn up and validated in order to enable the study to be conducted and monitored appropriately, thereby ensuring its quality. Whatever the type of

study, it is important that the person conducting the study has the appropriate qualifications, has training and experience in the field of the proposed study and has high ethical qualitied standards and professional integrity.

Test facilities should maintain a quality assurance system, including standardised operating procedures. A monitoring system should be set up for each study in order to ensure that the protocol and the operating procedures are correctly followed.

Data processing and the **interpretation of results** should be fair and should not overstep the limits of the test's significance. Data recording, transformations and representation in tabular or graphical form should be transparent or clearly explained if complex. It should not be designed to overstate the effect(s) measured. Appropriate statistical analysis of the data should be performed.

Appendix

Raw data from MTT results (OD 550 nm)

Control	MIX CNCE							
	3%	1%	0,30%	0,10%	0,03%	0,01%	0,003%	0,001%
0,892	0,429	0,680	0,761	0,795	0,749	0,852	0,801	0,984
0,926	0,377	0,663	0,783	0,789	0,789	0,886	0,812	0,955
0,869	0,349	0,623	0,698	0,783	0,743	0,869	0,841	0,921
0,909	0,320	0,646	0,663	0,772	0,806	0,869	0,869	0,932
0,995	0,297	0,583	0,721	0,749	0,789	0,858	0,869	0,812
1,012	0,320	0,623	0,646	0,778	0,783	0,841	0,949	0,881
1,132	0,486	0,635	0,726	0,783	0,783	0,858	0,972	0,961
1,092	0,526	0,738	0,829	0,926	0,761	0,863	0,978	0,863
1,012								
0,989								
1,001								
0,995								
1,018								
1,029								
1,041								
1,086								

Control	*Serenoa repens*							
	3%	1%	0,30%	0,10%	0,03%	0,01%	0,003%	0,001%
0,895	0,356	0,070	0,049	0,049	0,065	0,928	1,068	1,084
0,922	0,426	0,097	0,065	0,043	0,070	0,976	1,025	1,068
0,874	0,297	0,151	0,043	0,070	0,097	0,895	0,944	1,019
0,938	0,307	0,140	0,059	0,065	0,065	0,852	0,965	0,998
1,025	0,523	0,183	0,075	0,092	0,102	0,922	0,933	0,938
1,046	0,475	0,189	0,081	0,065	0,092	0,917	1,041	1,003
1,046	0,550	0,173	0,070	0,065	0,086	0,992	0,976	1,019
0,992	0,297	0,189	0,054	0,059	0,075	0,890	0,971	1,057
1,041								
1,052								
1,014								
1,030								
1,025								
1,008								
1,030								
1,062								

qPCR expression data after analysis

SRD5A1					
Control	**Control + (0.1 %)**	**MIX CNCE (0.001 %)**	**MIX CNCE (0.005 %)**	**Serenoa (0.001 %)**	**Serenoa (0.005 %)**
1	0,29	0,819	1,011	0,795	0,618
1,083	0,314	0,887	1,094	0,861	0,669
0,924	0,51	0,742	1,473	0,816	0,673
1	0,552	0,804	1,595	0,884	0,728
	1,019	1,048	1,278	1,205	0,679
	*1,103**	1,134	1,384	1,304	0,735
		0,818			0,925
		0,886			1,001

SRD5A2					
Control	**Control + (0.1 %)**	**MIX CNCE (0.001 %)**	**MIX CNCE (0.005 %)**	**Serenoa (0.001 %)**	**Serenoa (0.005 %)**
1,000	0,212	0,409	0,533	0,460	0,431
1,458	0,309	0,597	0,777	0,670	0,629
0,686	0,506	0,426	1,109	0,612	0,430
1,000	0,738	0,622	1,617	0,893	0,628
	0,888	0,638	0,657	0,857	0,477
	*1,294**	0,930	0,959	*1,249**	0,695
		0,473			0,568
		0,689			0,828

SRD5A3					
Control	**Control + (0.1 %)**	**MIX CNCE (0.001 %)**	**MIX CNCE (0.005 %)**	**Serenoa (0.001 %)**	**Serenoa (0.005 %)**
1,000	0,521	0,417	0,351	0,424	0,286
1,371	0,715	0,572	0,482	0,582	0,392
0,729	0,828	0,705	0,284	0,481	0,289
1,000	1,135	0,967	0,390	0,660	0,396
	1,019	0,463	0,432	0,803	0,438
	*1,397**	0,635	0,593	1,101	0,601
			0,361		0,496
			0,495		0,680

**Value excluded from the analysis, considered as outlier after technical processing of samples for RT-qPCR analysis..*

Raw data from qPCR before analysis

Sample Name	Target Name	RQ	CT
P. 1599 0.005% 1	ACTINA		16,112
P. 1599 0.005% 1	ACTINA		16,243
P. 1599 0.005% 1	ACTINA		16,264
P. 1599 0.005% 1	SRD5A1	0,623	23,965
P. 1599 0.005% 1	SRD5A1	0,623	24,312
P. 1599 0.005% 1	SRD5A2	0,434	27,593
P. 1599 0.005% 1	SRD5A2	0,434	27,512
P. 1599 0.005% 1	SRD5A3	0,288	24,597
P. 1599 0.005% 1	SRD5A3	0,288	24,854
P. 1599 0.005% 2	ACTINA		16,334
P. 1599 0.005% 2	ACTINA		16,290
P. 1599 0.005% 2	ACTINA		16,246
P. 1599 0.005% 2	SRD5A1	0,677	23,854
P. 1599 0.005% 2	SRD5A1	0,677	23,992
P. 1599 0.005% 2	SRD5A1	0,677	24,459
P. 1599 0.005% 2	SRD5A2	0,433	27,650
P. 1599 0.005% 2	SRD5A2	0,433	27,630
P. 1599 0.005% 2	SRD5A3	0,290	24,766
P. 1599 0.005% 2	SRD5A3	0,290	24,779
P. 1599 0.005% 2	SRD5A3	0,290	24,847
P. 1599 0.005% 3	ACTINA		16,427
P. 1599 0.005% 3	SRD5A1	0,686	24,524
P. 1599 0.005% 3	SRD5A1	0,686	23,956
P. 1599 0.005% 3	SRD5A1	0,686	24,180
P. 1599 0.005% 3	SRD5A2	0,482	27,523
P. 1599 0.005% 3	SRD5A2	0,482	27,732
P. 1599 0.005% 3	SRD5A2	0,482	27,614
P. 1599 0.005% 3	SRD5A3	0,443	24,291
P. 1599 0.005% 3	SRD5A3	0,443	24,361
P. 1599 0.005% 4	ACTINA		16,880
P. 1599 0.005% 4	ACTINA		16,435
P. 1599 0.005% 4	ACTINA		16,696
P. 1599 0.005% 4	SRD5A1	0,935	24,093
P. 1599 0.005% 4	SRD5A1	0,935	23,951
P. 1599 0.005% 4	SRD5A1	0,935	24,008
P. 1599 0.005% 4	SRD5A2	0,574	27,667
P. 1599 0.005% 4	SRD5A2	0,574	27,563
P. 1599 0.005% 4	SRD5A3	0,501	24,419
P. 1599 0.005% 4	SRD5A3	0,501	24,365
P. 1599 0.001% 1	ACTINA		16,850
P. 1599 0.001% 1	ACTINA		16,805
P. 1599 0.001% 1	SRD5A1	0,838	24,313
P. 1599 0.001% 1	SRD5A1	0,838	24,350
P. 1599 0.001% 1	SRD5A2	0,485	28,096
P. 1599 0.001% 1	SRD5A2	0,485	27,892
P. 1599 0.001% 1	SRD5A2	0,485	28,060
P. 1599 0.001% 1	SRD5A3	0,447	24,713
P. 1599 0.001% 2	ACTINA		16,896
P. 1599 0.001% 2	SRD5A1	0,831	24,398
P. 1599 0.001% 2	SRD5A1	0,831	24,368
P. 1599 0.001% 2	SRD5A1	0,831	24,472
P. 1599 0.001% 2	SRD5A2	0,623	27,716
P. 1599 0.001% 2	SRD5A2	0,623	27,896
P. 1599 0.001% 2	SRD5A2	0,623	27,549
P. 1599 0.001% 2	SRD5A3	0,490	24,650
P. 1599 0.001% 3	SRD5A1		23,968
P. 1599 0.001% 3	SRD5A1		24,157
P. 1599 0.001% 3	SRD5A2		27,340
P. 1599 0.001% 3	SRD5A2		27,065
P. 1599 0.001% 3	SRD5A2		27,252
P. 1599 0.001% 3	SRD5A3		23,844
P. 1599 0.001% 3	SRD5A3		24,048
P. 1599 0.001% 3	SRD5A3		24,223
P. 1599 0.001% 4	ACTINA		16,995
P. 1599 0.001% 4	ACTINA		17,113
P. 1599 0.001% 4	ACTINA		17,218
P. 1599 0.001% 4	SRD5A1	1,214	24,135
P. 1599 0.001% 4	SRD5A1	1,214	24,021
P. 1599 0.001% 4	SRD5A2	0,863	27,554
P. 1599 0.001% 4	SRD5A2	0,863	27,519
P. 1599 0.001% 4	SRD5A2	0,863	27,318
P. 1599 0.001% 4	SRD5A3	0,809	24,063
P. 1599 0.001% 4	SRD5A3	0,809	24,251
P. 1599 0.001% 4	SRD5A3	0,809	24,103
P. 1598 0.005% 1	ACTINA		17,140
P. 1598 0.005% 1	ACTINA		17,202
P. 1598 0.005% 1	SRD5A1	1,005	24,402
P. 1598 0.005% 1	SRD5A1	1,005	24,445
P. 1598 0.005% 1	SRD5A1	1,005	24,390
P. 1598 0.005% 1	SRD5A2	0,530	28,159
P. 1598 0.005% 1	SRD5A2	0,530	28,215
P. 1598 0.005% 1	SRD5A2	0,530	28,313
P. 1598 0.005% 1	SRD5A3	0,415	25,165
C1	ACTINA		17,177
C1	ACTINA		17,022
C1	ACTINA		17,369
C1	SRD5A1	1,000	24,458
C1	SRD5A1	1,000	24,419
C1	SRD5A2	1,000	27,484
C1	SRD5A2	1,000	27,324
C1	SRD5A2	1,000	27,189
C1	SRD5A3	1,000	23,851
C1	SRD5A3	1,000	23,975
P. 1598 0.005% 2	ACTINA		17,728
P. 1598 0.005% 2	ACTINA		17,791
P. 1598 0.005% 2	SRD5A1	1,505	24,419
P. 1598 0.005% 2	SRD5A2	1,133	27,799
P. 1598 0.005% 2	SRD5A2	1,133	27,661

P. 1598 0.005% 2	SRD5A2	1,133	27,707
P. 1598 0.005% 2	SRD5A3	0,721	24,948
P. 1598 0.005% 2	SRD5A3	0,721	24,852
P. 1598 0.005% 2	SRD5A3	0,721	25,068
C2	SRD5A1		25,327
C2	SRD5A1		25,478
C2	SRD5A2		28,819
C2	SRD5A2		28,803
C2	SRD5A3		25,101
C2	SRD5A3		25,153
C2	SRD5A3		24,969
P. 1598 0.005% 3	ACTINA		16,748
P. 1598 0.005% 3	ACTINA		16,815
P. 1598 0.005% 3	ACTINA		16,824
P. 1598 0.005% 3	SRD5A1	1,293	23,693
P. 1598 0.005% 3	SRD5A1	1,293	23,655
P. 1598 0.005% 3	SRD5A2	0,665	27,816
P. 1598 0.005% 3	SRD5A2	0,665	27,212
P. 1598 0.005% 3	SRD5A2	0,665	27,553
P. 1598 0.005% 3	SRD5A3	0,468	24,560
P. 1598 0.005% 3	SRD5A3	0,468	24,683
P. 1598 0.005% 3	SRD5A3	0,468	24,601
C3	ACTINA		17,013
C3	ACTINA		16,874
C3	SRD5A1	0,977	24,247
C3	SRD5A1	0,977	24,201
C3	SRD5A1	0,977	24,230
C3	SRD5A2	0,725	27,485
C3	SRD5A2	0,725	27,614
C3	SRD5A3	0,771	24,030
C3	SRD5A3	0,771	24,054
P. 1598 0.005% 4	SRD5A1		23,922
P. 1598 0.005% 4	SRD5A1		23,853
P. 1598 0.005% 4	SRD5A2		27,602
P. 1598 0.005% 4	SRD5A2		27,385
P. 1598 0.005% 4	SRD5A2		27,272
P. 1598 0.005% 4	SRD5A3		24,166
P. 1598 0.005% 4	SRD5A3		24,151
P. 1598 0.005% 4	SRD5A3		24,193
C+ 1	ACTINA		16,984
C+ 1	SRD5A1	0,309	25,923
C+ 1	SRD5A1	0,309	25,934
C+ 1	SRD5A2	0,225	29,224
C+ 1	SRD5A2	0,225	29,330
C+ 1	SRD5A3	0,555	24,663
C+ 1	SRD5A3	0,555	24,450
P. 1598 0.001% 1	ACTINA		16,165
P. 1598 0.001% 1	ACTINA		16,367
P. 1598 0.001% 1	SRD5A1	0,828	23,712
P. 1598 0.001% 1	SRD5A1	0,828	23,800
P. 1598 0.001% 1	SRD5A1	0,828	23,847
P. 1598 0.001% 1	SRD5A2	0,414	27,718
P. 1598 0.001% 1	SRD5A2	0,414	27,645
P. 1598 0.001% 1	SRD5A3	0,355	24,619
P. 1598 0.001% 1	SRD5A3	0,355	24,483
P. 1598 0.001% 1	SRD5A3	0,355	24,346
C+ 2	ACTINA		17,556
C+ 2	SRD5A1	0,532	25,462
C+ 2	SRD5A1	0,532	25,721
C+ 2	SRD5A1	0,532	25,968
C+ 2	SRD5A2	0,527	28,676
C+ 2	SRD5A2	0,527	28,570
C+ 2	SRD5A3	0,862	24,430
C+ 2	SRD5A3	0,862	24,562
C+ 2	SRD5A3	0,862	24,487
P. 1598 0.001% 2	ACTINA		16,297
P. 1598 0.001% 2	ACTINA		16,350
P. 1598 0.001% 2	SRD5A1	0,766	23,902
P. 1598 0.001% 2	SRD5A1	0,766	24,013
P. 1598 0.001% 2	SRD5A2	0,440	27,661
P. 1598 0.001% 2	SRD5A2	0,440	27,642
P. 1598 0.001% 2	SRD5A3	0,293	24,756
P. 1598 0.001% 2	SRD5A3	0,293	24,833
P. 1598 0.001% 2	SRD5A3	0,293	24,859
C+ 3	SRD5A1		25,227
C+ 3	SRD5A1		25,190
C+ 3	SRD5A1		25,383
C+ 3	SRD5A2		28,313
C+ 3	SRD5A2		28,407
C+ 3	SRD5A3		24,786
C+ 3	SRD5A3		24,750
C+ 3	SRD5A3		24,691
P. 1598 0.001% 3	ACTINA		16,925
P. 1598 0.001% 3	ACTINA		16,843
P. 1598 0.001% 3	SRD5A1	1,082	24,009
P. 1598 0.001% 3	SRD5A1	1,082	24,031
P. 1598 0.001% 3	SRD5A2	0,659	27,629
P. 1598 0.001% 3	SRD5A3	0,446	24,766
P. 1598 0.001% 3	SRD5A3	0,446	24,861
P. 1598 0.001% 3	SRD5A3	0,446	24,688
P. 1598 0.001% 4	ACTINA		16,230
P. 1598 0.001% 4	ACTINA		16,229
P. 1598 0.001% 4	SRD5A1	0,840	23,750
P. 1598 0.001% 4	SRD5A1	0,840	23,711
P. 1598 0.001% 4	SRD5A2	0,485	27,456
P. 1598 0.001% 4	SRD5A2	0,485	27,377
P. 1598 0.001% 4	SRD5A3	0,371	24,363
P. 1598 0.001% 4	SRD5A3	0,371	24,472
P. 1598 0.001% 4	SRD5A3	0,371	24,320