EX VIVO ASSESSMENT OF THE EFFICACY OF CYNATINE® TOP, A COSMETIC INGREDIENT, IN PROTECTING HAIR STRUCTURE FROM UV EXPOSURE

KERAT'INNOV

CYNATINE® TOP

Complife Italia S.r.l.
info@complifegroup.com
PEC complifeitalia@legalmail.it
complifegroup.com

Sede di Garbagnate Milanese (MI):
Via Guido Rossa, 1
20024 Garbagnate M.se (MI) Italy
+39.02.990.25138
+39.02.990.25007

Sede di S.Martino Siccomario (PV)
Via Monsignor Angelini, 21,
27028 S.Martino Siccomario (PV)
+39.0382.25504
+39.0382.536006

Sede legale: Via Guido Rossa, 1 20024 Garbagnate M.se (MI) Italy, | P.I./C.F. 11093320155
Capitale Sociale € 161.834 i.v., C.C.I.A.A. MI 1437929 - Registro Società N. 342215.
Centro di Saggio operante in BPL - Sistema di gestione qualità certificato ISO 9001

KEY PERSONNEL

Customer

KERAT'INNOV
37, Avenue du Mistral
13600 La Ciotat (France)

Investigator

Dr **Gioia Bizzaro**
Biologist
Complife Italia S.r.l.

Quality control

Dr **Vincenzo Nobile**
Biologist
Complife Italia S.r.l.

Complife Italia S.r.l

Location
Via Angelini, 21
27028 San Martino Siccomario (PV)
Italy
tel. +39-0382 25504 - fax +39-0382 536006
Mail: info@complifegroup.com

TABLE OF CONTENTS

SECTION 1 - STUDY DESIGN

1.1. Title

Ex vivo assessment of the efficacy of CYNATINE® TOP, a cosmetic ingredient, in protecting hair structure from uv exposure.

1.2. Aim

The study is aimed to assess the efficacy of a cosmetic ingredient in protecting hair structure from UV exposure. In order to reach this goal an *ex vivo* study is carried out on commercially available natural origin human hair locks. Product efficacy is assessed by means of biochemical analysis and expert assessment.

1.3. Tested products

1.3.1. Information provided by the Customer

☒ Products identification

Product name	batch. no.	CAS
CYNATINE® TOP	K170144-8	69430-36-0

☒ The tested cosmetic products conform to Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast) (Text with EEA relevance) and to its annexes.

☒ How to use: use the ingredient at 0.5% concentration.

☒ Qualitative INCI formula: HYDROLYZED KERATIN

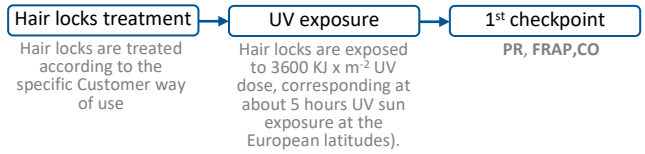
1.4. MATERIALS & METHODS

In the sections here below are reported the materials and the methods employed in the study.

1.4.1. Study flow and schedule of assessments chart

The study flow and the schedule of assessments chart is reported in Figure 1.4.1.1.

Figure 1.4.1.1 Study flow and schedule of assessments chart.



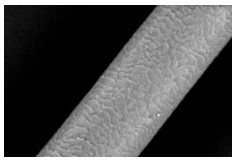
Legend. **PR** protein content assay | **FRAP** antioxidant potential | **CO** Combing

1.4.2. Hair locks

For this study commercially available hair locks of human origin are used. The hair lock has a morphology both macro- and microscopic similar to that of the human hairs *in vivo*. Table 1.4.2.1 shows hair locks characteristics.

Table 1.4.2.1 Hair locks characteristics.

Length:	≈ 15 cm
Color:	Brown
Appearance:	Straight
Consistence:	Natural
Weight:	10 grams



The image shows an example of SEM picture (1 KX) of the hairs' lock.

The image shows an example of macroscopic morphology of the hairs lock.

1.4.3. Hair locks preparation and treatment

After their receipt the hair locks are divided in 10 locks of 10 grams each and washed using a neutral shampoo. After the washing procedure the hair locks are slightly bumped with a towel and then dried with an hairdryer. After drying, five (n=5) hair locks are immersed in a 0.5% water solution of CYNATINE® TOP and five (n=5) hair locks are immersed only in water (negative control). Hair locks are then slightly bumped with a towel and then dried with an hairdryer. After drying hair locks are exposed to UV using a solar simulator.

1.4.4. Exposure to UV

Hair locks are exposed to UV radiation using a Sun test CPS+ (Atlas Material Testing Technology, USA. Fig. 1.4.4.2.1) solar simulator. The UV dose applied to the hair locks was 3600 KJ·m^{-2}. The dose is delivered in 2 hours exposure at 1800 KJ·m^{-2}. The chosen dose corresponds at about 5 hours exposure to the sun at the European latitudes.

Figure 1.4.4.2.1 Solar simulator characteristics. The artificial UV irradiation source consists of a Suntest CPS+ (Atlas). The energetic irradiance of the instrument is reported in the table here below.

Model	Atlas Suntest CPS+
Lamp	1x1500 W Xenon Lamps
Exposure area	560 cm^2
Benchtop design	90x35x35 cm

Spectrum region	Energetic irradiance, $W \cdot m^{-2}$
<290 nm	0.125
dUVB (290-320 nm)	5.68
dUVA (320-400 nm)	75.2
UVA-2 (320-340 nm)	10.93
UVA-1 (340-400 nm)	64.25
UV (290-400 nm)	80.75
VIS (400-780 nm)	535
IR-A (780-1400 nm)	741.5
Total (UV+VIS+IR-A)	1357

Legend
Irradiance, Intensity of radiation per unit surface falling (from a source) onto a test surface ($W \cdot m^{-2}$)

The UV irradiance falls with the following acceptance limits: dUVA/dUVB irradiances from 8 to 22 (13.2), total UV irradiance from 40-200 $W \cdot m^{-2}$ (80.9).

1.5.5. Endpoints

For biochemical analysis, hair locks are solubilised overnight in a 37% HCl solution (ACS reagent, CAS NO. 7647-01-0, MW 36.46, code 288148-2.5L), as follows: i) weigh 1 gram of untreated/ treated hair in a 50 ml vial using an analytical balance (KERN ALJ 160-4NM, KERN & Sohn GmbH), ii) add 3 ml of a 37% HCl solution and mix well in order to ensure that hair are complete immersed in the acid solution, iii) incubate overnight in a thermostated water bath (GRANT GLS400, Grant Instruments) at 50°C under continuous agitation, and iv) dilute the obtained digestate 1:10 using distilled water before to carry out the biochemical assays.

1.5.5.1. Total antioxidant capacity

The hair ability to resist the damage induced by free radicals is measured by means of the Ferric Reducing Antioxidant Power (FRAP) assay. FRAP uses the antioxidants in the biological system as reductive agent in a colorimetric method based on redox reactions [Benzie IFF, Strani JJ, Anal Biochem 1996, 239: 70-76].

$$\text{TPTZ-Fe(III)} \xrightarrow[\text{pH = 3.6 T= 37° C}]{\text{Antioxidants}} \text{TPTZ-Fe(II)}$$

The reduction at acid pH of the complex TPTZ-Fe(III) in the ferrous form (Fe(II)) is characterized by an intense blue color. The reaction is monitored by measuring the solution absorbance at 595 nm. The recorded absorbance is compared to a Fe(II) standard curve of known values. The results is directly proportional to the total reductive power of the antioxidant in the reaction mix.

1.5.5.2. Protein content

Total protein content is measured according to Lowry method (Lowry OH, Rosebrough NJ, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem, 1951 Nov;193(1):265-75).

1.5.5.3. Combing

The investigator counts the number of comb passes needed to perfectly detangle the hair.

1.6. Results and statistic

1.6.1. Results

1) Results are reported in tables in their respective units.
2) Mean values are calculated as:

$$m = \frac{\sum_{1}^{n} p}{n}$$

where: p is the value of the parameter under analysis; n is the number of subjects participating in the study

3) Percentages are calculates as follows:

$$\%\text{var.vs. T0} = \left(\sum_{1}^{n} \frac{p_t - p_0}{p_0} \right) x100$$

where: p_t is the value of the parameter under analysis after product application; p_0 is the value of the parameter under analysis before product application; n is the number of subjects participating in the study

4) The mean standard error of data is calculated as:

$$SE. = \frac{\sqrt{\sum_{1}^{n}(p_i^2) - \frac{\sum_{1}^{n} p_i^2}{n}}{(n-1)}}{\sqrt{n}}$$

where: p is the value of the parameter under; n is the number of subjects participating in the study

All the calculations are done using a Microsoft® Excel 2013 (vers. 15.0.4953.1001; Microsoft, USA) worksheet running on Microsoft® Windows 8.1 Professional (Microsoft, USA).

1.7. Report change record

The table here below reports the change log of all approved changes made to the document that make up the course after initial approval.

Rev. no	Date	Description
00	25/05/2018	First release
01	29/05/2018	Minor modification

RESULTS

Table 1. The table here below summarizes the data obtained during the study. Data are reported as mean ± std. dev. in their respective units. In bracket is reported the % variation vs. untreated hair locks.

	Untreated hair locks		CYNATINE® TOP treated hair locks		
	Before	**After**	**Before**	**After**	
FRAP	---	572.8±38.4	---	644.9±42.2 **(+12.6%)**	µM Fe^{2+}
Protein content	---	88.2±8.5	---	105±7.1 **(+19.1%)**	µg
Combing	6.2±0.8	10.4±1.1	7.2±0.8	7.6±1.5	no.

As it is possible to notice **CYNATINE® TOP**:

- ✓ preserves and determines an increase in hair protein content and in hair antioxidant capacity when compared to untreated hair exposed to UV;
- ✓ preserves hair combing after UV exposure.

Investigator

Dr *Gioia* BIZZARO

Digitally signed by Gioia Bizzaro
Date: 2018.05.29 09:57:41 +02'00'

Quality control

Dr *Vincenzo* NOBILE

Digitally signed by Vincenzo Nobile
Date: 2018.05.29 15:13:08 +02'00'