BIO+ INGREDIENTS

Natural Ingredients with Bioavailability Improvement

Nutrae by layn®

INNOVATION

New Product Development

Layn Europe s.r.l.

INNOVATION

BIO+
INNOVATION

NUTRAE
by LAYN

BIO+ Technology

GREEN TECHNOLOGY

✓Mesoporous silica is an important technology in the functioning of active ingredients for Nutraceutical Delivery Systems
due to its **beneficial effect on the stability and solubility.**

✓BIO+ technology with mesoporous silica is obtained by a **patented process**.

✓**In vitro to in vivo correlation (IVIVC) studies** follow to demonstrate the benefits for human health thanks to bioavailability improvement.



Advantages

Compliance (size/posology) -> reduced dosage of active principle
Compliance (colorless/tasteless/odorless)

+ solid formulation
+ only active ingredient & BIO+ mesoporous silica
+ solvent-free green process

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INNOVATION

NEW TECHNOLOGY

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Need for New Technologies

Silica Drug Delivery

Most used advanced solubilization technology approaches

01 Particle Size Reduction

02 Complexation Systems

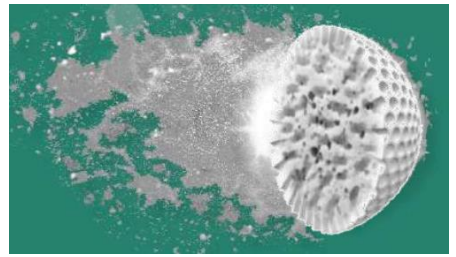
03 Lipid-based Systems

04 Polymers & Solid Dispersions

- Each technology approach has disadvantages
- No one technology works on all components
- Number of poorly soluble compounds increasing rapidly

Conclusion:
Combination of technologies with additional synergistic effects

05 New Silica based delivery system



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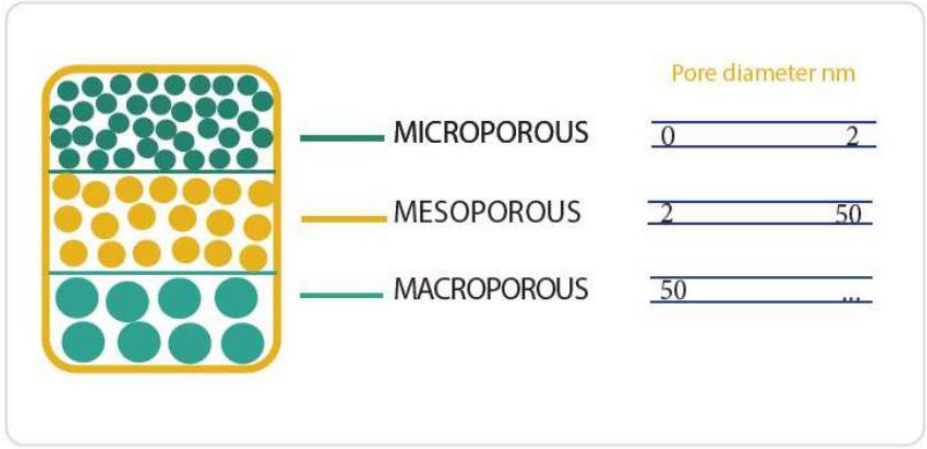
INNOVATION

MESOPOROUS SILICA

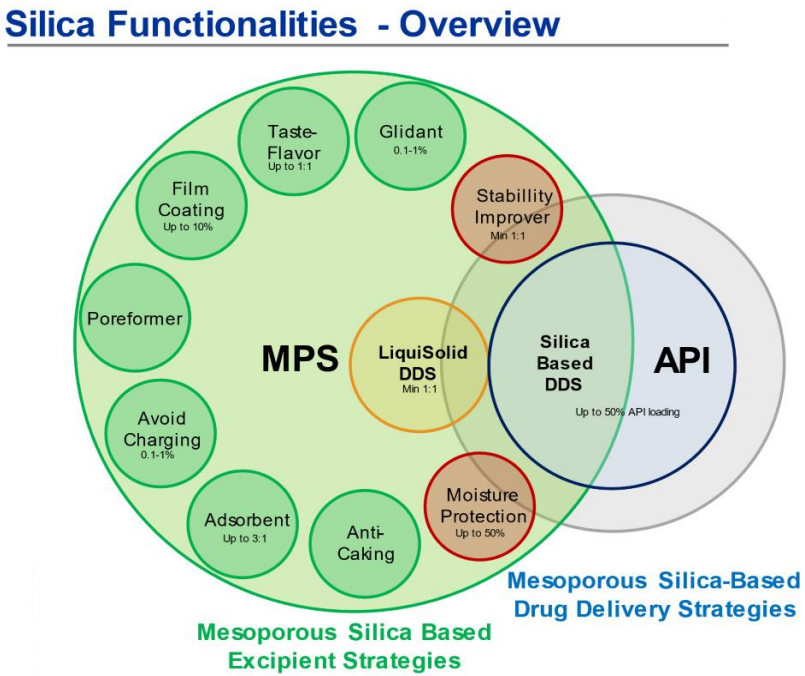
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What is MESOPOROUS SILICA

-overview-



- Contains 3D and periodic arrangements of atoms
- Structures with holes (pores)
- Micron size particles (not nanometric)
- Synthetic Amorphous Silicon Dioxide (non-crystalline)



Reference:
Choudari et al., Advances in Delivery Science and Technology 2014, pp.665-633

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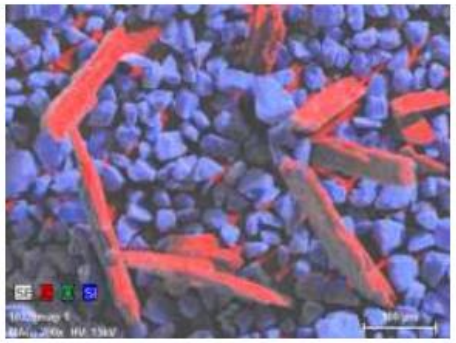
INNOVATION

GREEN TECHNOLOGY

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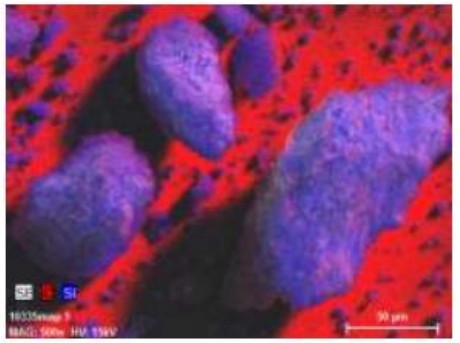
SILICA DRUG DELIVERY

Mesoporous Silica assisted Solvent Free Green Technology



Silica Particles and Crystalline API Pre-blend Mix

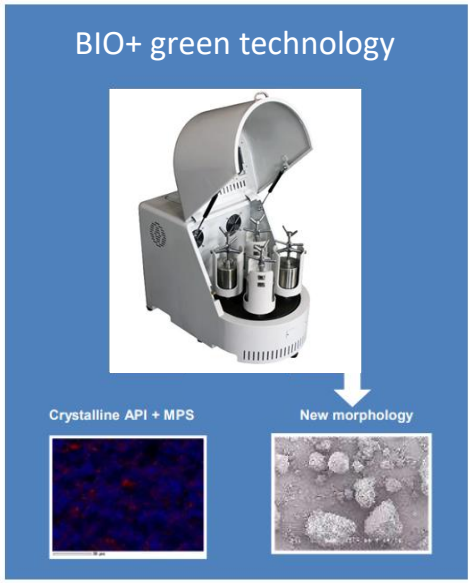
BIO+ TECHNOLOGY



Silica Active Sites Help Induce and Stabilize Amorphous Forms

API RELEASED IN GI TRACT

Loading Silica using **solvent free** technology



Crystalline API + Mesoporous Silica

↓

Right Energy & Affinity

↓

Melting

↓

Amorphous Free Flow Powder

s:

[1] Watanabe et al. 2001 + Bogner et al. 2002: Milling Induces Reactions between API (IMC) and the Silanol Groups.

[2] Jarowski et al. 1978: Milling Induces Reactions between API and the Silanol Groups. SYLOID® FP silica

[3] Riikka Laitinen, Thomas Rades et al. 2012: Milling induced reactions are due to Hydrogen Bonding with Silanol Groups

[4] Lauretta Maggi, Andrea Canobbio et al. 2015: SYLOID® FP silicas cause significant enhancement in solubility

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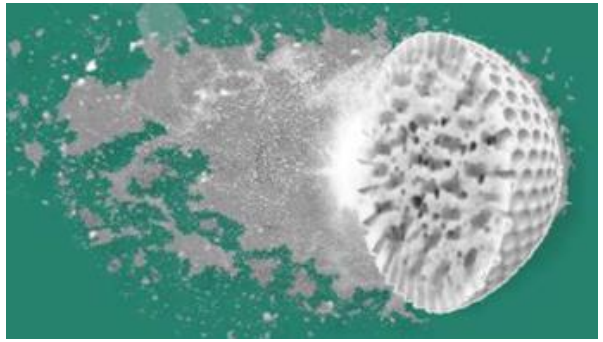
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PATENTED TECHNOLOGY

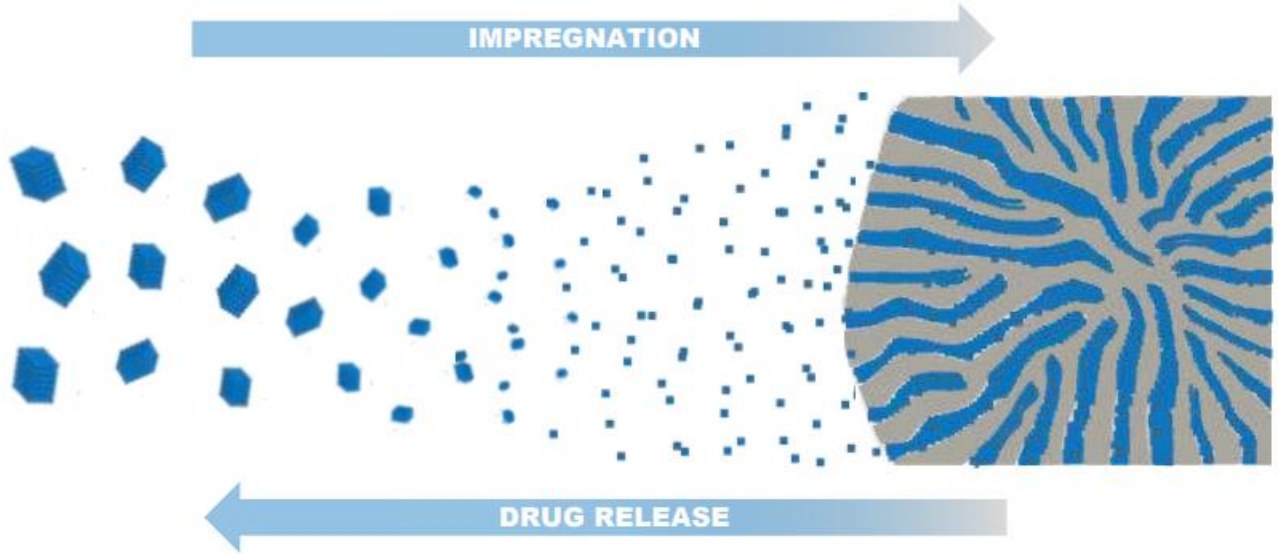
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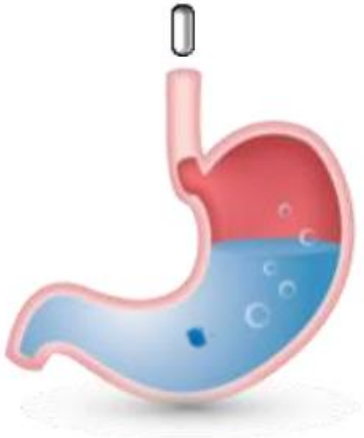
HOW IT WORKS



- A stable amorphous phase results from confinement of the active ingredient in pores of sub-critical dimensions and from the strength of the absorptive interaction (H-bonding)
- On contact with gastric fluids, the confined amorphous drug is rapidly released







References:
[1] Mellaerts et al. Chem Commun (13):1375–1377, 2007
[2] Van Speybroeck et al. 2009. J Pharm Sci 98(8):2648–2658.

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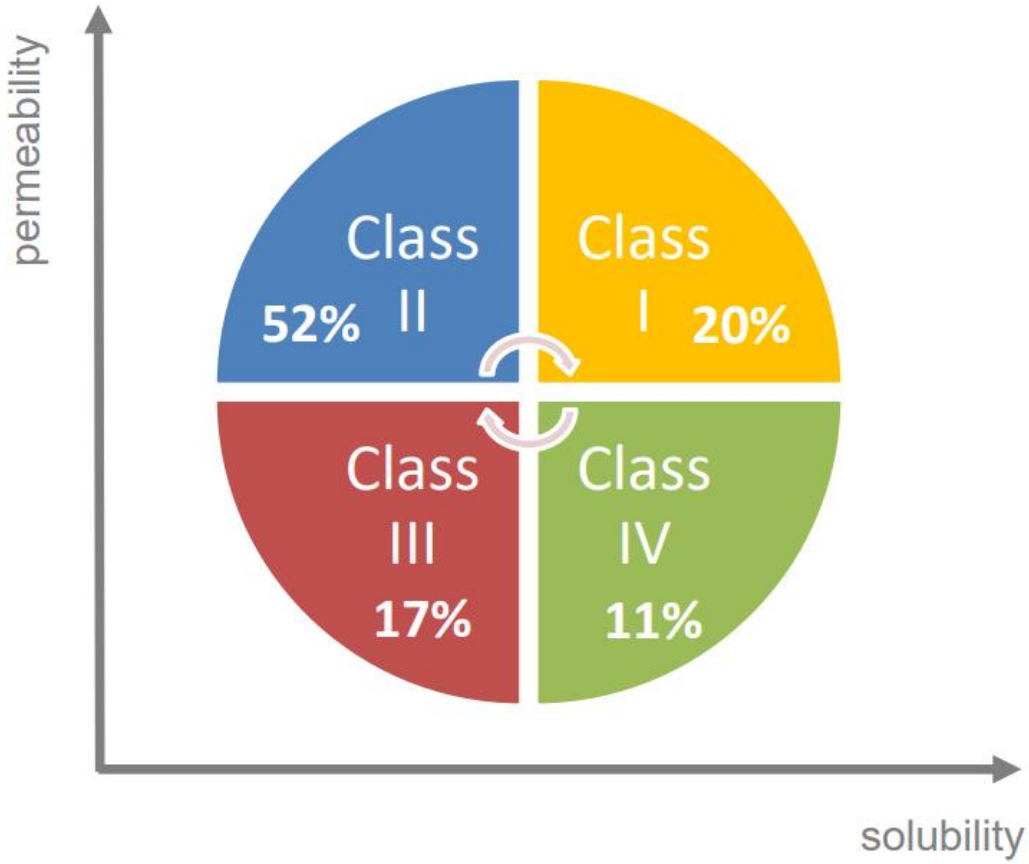
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INNOVATION

LOW SOLUBILITY ISSUE

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Biopharmaceutical Classification System (BCS)



Class II

Low solubility

in Gastrointestinal Tract is the main issue of

NATURAL ACTIVE INGREDIENTS

References:
[1] Amidon et al., Pharm. Res. 12 (1995)
[2] Sedo, PharmaCiRcle, APV conference (2016)

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BIO+Resveratrol®

Trans-Resveratrol with enhanced bioavailability

Nutrae by layn®

INNOVATION

bio+Resveratrol

ENHANCED BIOAVAILABILITY

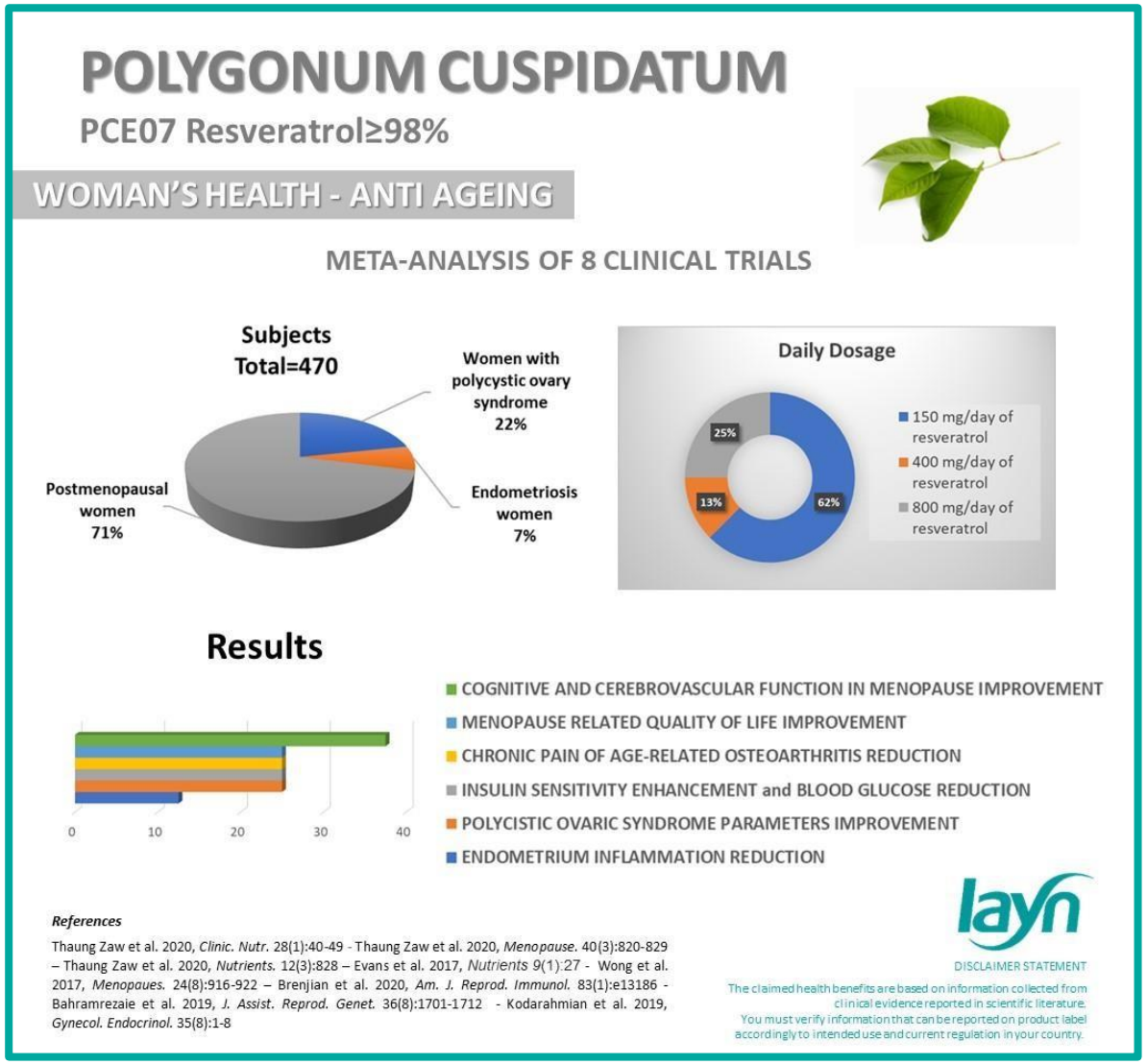
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RESVERATROL Potential & Issue

NUTRAE by LAYN

RESVERATROL



But…

Oral bioavailability of pure resveratrol in the human body seems to be less than 1%.

High dosages of resveratrol lead to side effects (as flatulence, gastric irritation)

References:
[1] Walle, 2011, Ann. N. Y. Acad. Sci.;1215:9-15, Bioavailability of resveratrol
2 Wenzel and Somoza, 2005 Metabolism and bioavailability of trans-resveratrol
3Walle et al. 2004, High absorption but very low bioavailability of oral resveratrol in humans

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INNOVATION

BIO+Resveratrol®

NUTRAE by LAYN

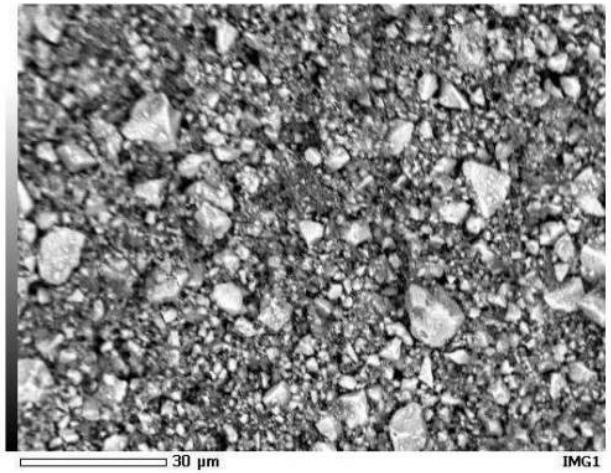
bio+Resveratrol

ENHANCED BIOAVAILABILITY

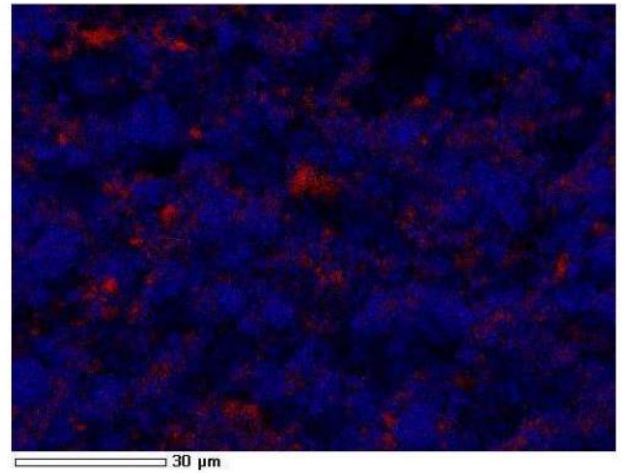
- BIO+Resveratrol® is a resveratrol-based solid dispersion with mesoporous silica as carrier
- BIO+Resveratrol® is obtained by milling process where resveratrol particles size are reduced by at least factor 10
- In BIO+Resveratrol®, resveratrol particles are dispersed on all the surface of the mesoporous silica
- The BIO+ green technology process led to a decrease of the number of resveratrol particles agglomerates



Morphology of resveratrol pure crystalline material by SEM. Particles of several hundred of microns



Morphology of BIO+Resveratrol® by SEM.



EDX characterization performed with Si (blue) and carbon (red) probes on the surface of the materials shown on SEM photo.

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INNOVATION

BIO+Resveratrol®

In Vitro Study

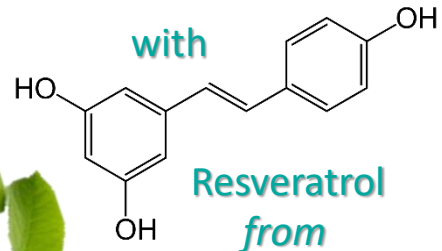
NUTRAE by LAYN

In Vitro

bio+Resveratrol

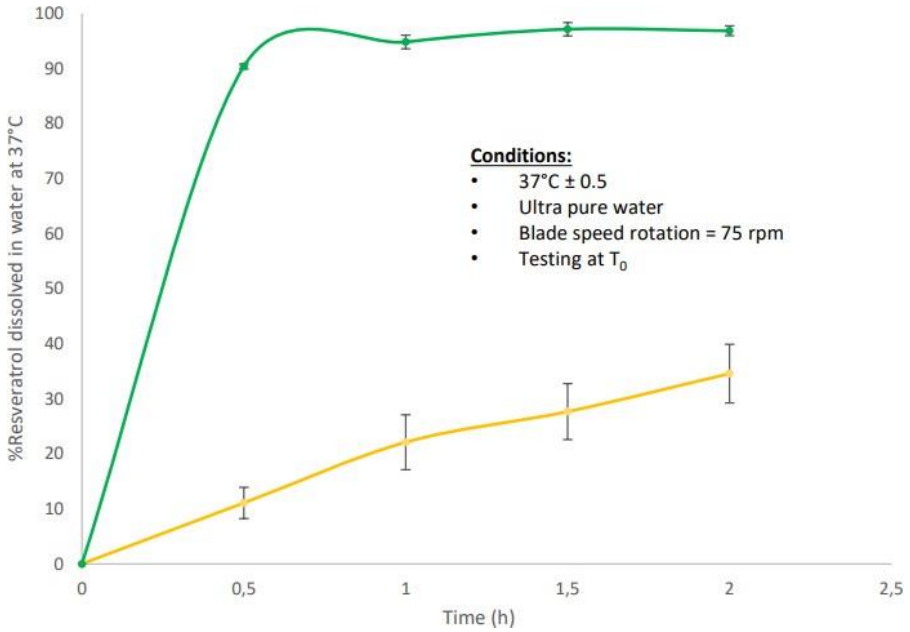
ENHANCED BIOAVAILABILITY

with



Resveratrol *from Polygonum Cuspidatum* PCE07

In Vitro Study



Graph:
Resveratrol dissolution kinetics expressed in % of dissolution for Resveratrol pure material PCE07 (**yellow curve**) and resveratrol of BIO+RESVERATROL (**green curve**).

The study highlights that resveratrol solid dispersion with mesoporous silicas **BIO+RESVERATROL significantly increases the dissolution kinetics of resveratrol.**
More precisely, the resveratrol dissolution of BIO+RESVERATROL in water at 37°C **is increased by a factor 8,2 (720%) after 30 min trial and by factor 3,2 (220%) after 2 hours** compared to pure resveratrol.

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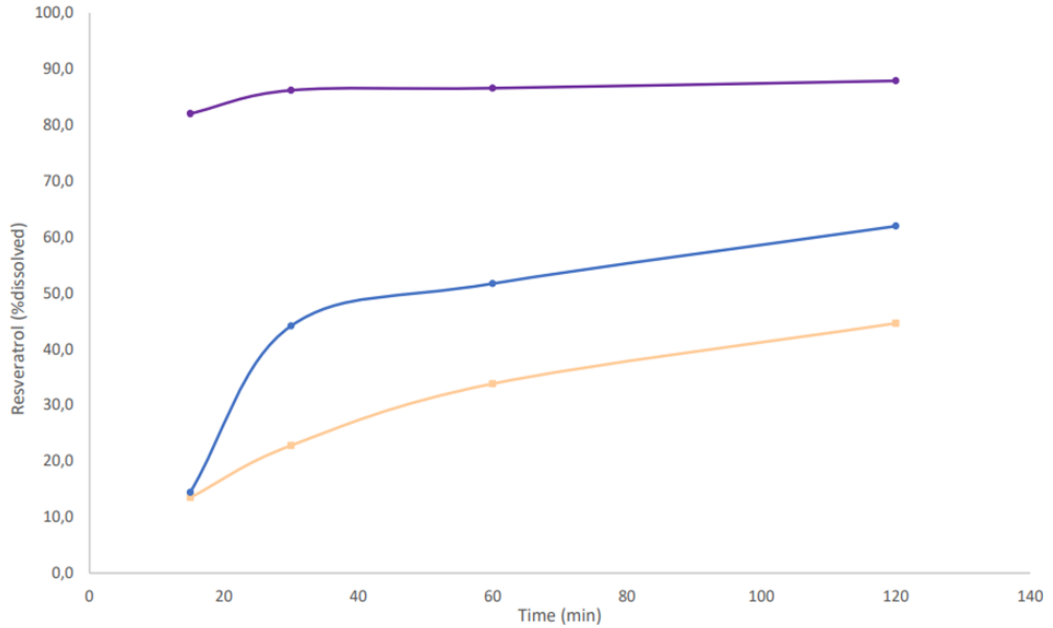
INNOVATION

In Vitro Study: Silica Advantages

NUTRAE by LAYN

In Vitro

SILICA EFFECT on BIO+Resveratrol®
in-vitro resveratrol release



Graph:
Resveratrol dissolution kinetics for resveratrol pure (**orange curve**), resveratrol milled (**blue curve**) and resveratrol of BIO+RESVERATROL (**purple curve**).

Only the reduction of the resveratrol particle size by ball milling does not allow to obtain such a high dissolution kinetics of resveratrol as the one obtained with the solid dispersion based on mesoporous silica as a carrier.

The presence of mesoporous silica as carrier is mandatory in order to reach such high dissolution kinetics of resveratrol.

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INNOVATION

BIO+Resveratrol®
In Vivo Study

NUTRAE
by LAYN

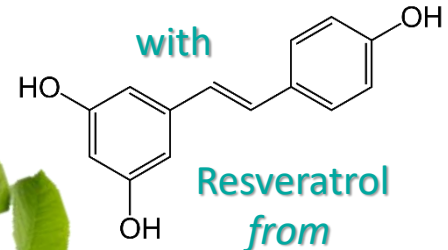


In Vivo

bio+Resveratrol

ENHANCED BIOAVAILABILITY

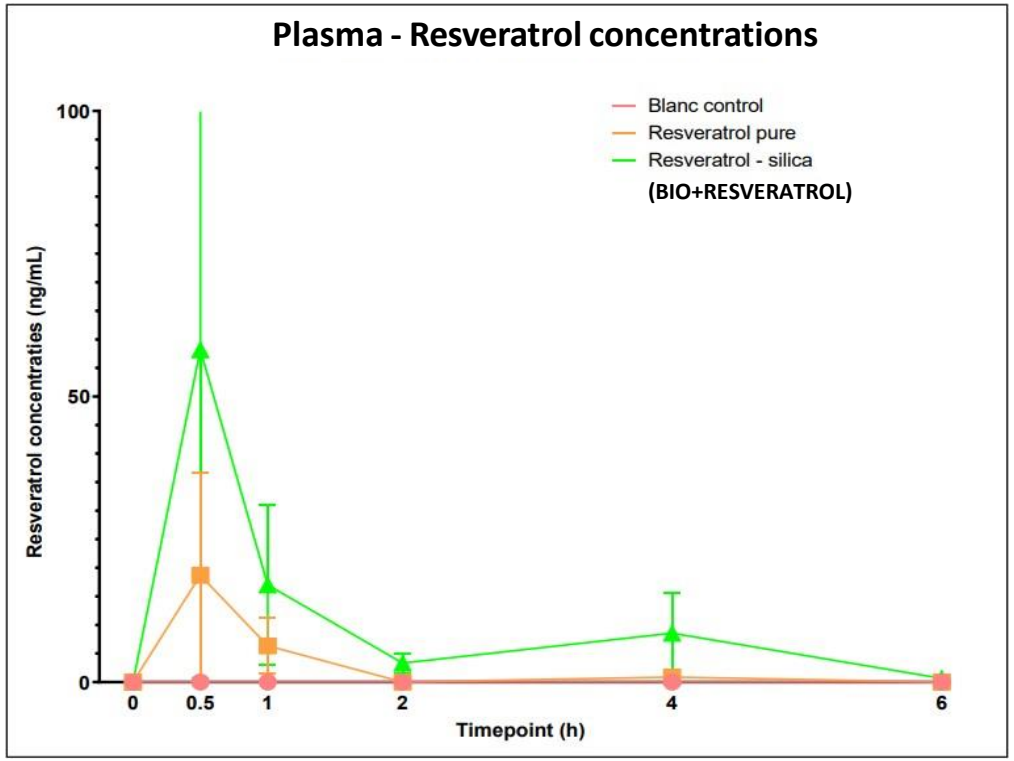
with



Resveratrol *from* *Polygonum Cuspidatum* PCE07



In Vivo Pre-Clinical Study



Graph:
Area Under the Curve (AUC) defined by concentration of resveratrol in blood plasma of rats, after oral ingestion, as a function of time.
In pharmacokinetics, AUC is used to determine bioavailability.

Pharmacokinetic calculations based on AUC in rats' plasma show resveratrol of BIO+Resveratrol® to have

4 TIMES (300%) BIOAVAILABILITY IMPROVEMENT

compared to pure resveratrol.

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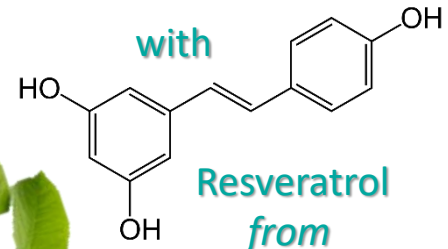
BIO+Resveratrol®
Human Clinical Trial

NUTRAE by LAYN

Clinical

bio+Resveratrol
ENHANCED BIOAVAILABILITY

with



Resveratrol *from* *Polygonum Cuspidatum* PCE07

Human Clinical Trial Protocol

- **Trial Objective**
  To evaluate the X-times improved plasma concentration of resveratrol formulated into Bio+Resveratrol® whereby a reduced % of Resveratrol can be proposed
- **Study Design**
  Single centre, prospective, randomized non-controlled study
- **Enrolment**
  12 healthy volounteers (aged 23-56 y.o.) for each group
- **Supplementation**
  Group 1 (n=12) : 125 mg of standard resveratrol (≥98% purity)
  Group 2 (n=12): 125 mg of resveratrol 30% loaded into mesoporous silicium dioxide
  Single Oral Intake in Capsule with a glass of 200mL water
- **Samples collection**
  T_0 (before intake of capsule) - $T_{0,30h}$ - T_{1h} - T_{2h} (after T 2hr a light lunch is possible) - T_{3h} - T_{4h} -T_{6h}
- **Analysis**
  Extraction of Resveratrol from human plasma samples has been performed using protein precipitation and followed by analysis using an LC-MS/MS .

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BIO+Resveratrol®
Human
Clinical Trial

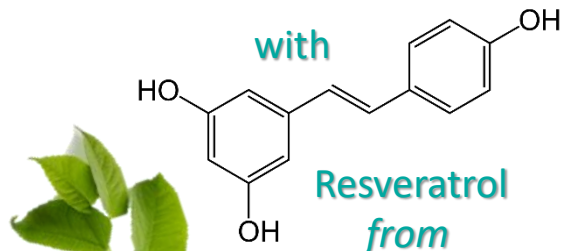
NUTRAE
by LAYN

Clinical

bio+Resveratrol

ENHANCED BIOAVAILABILITY

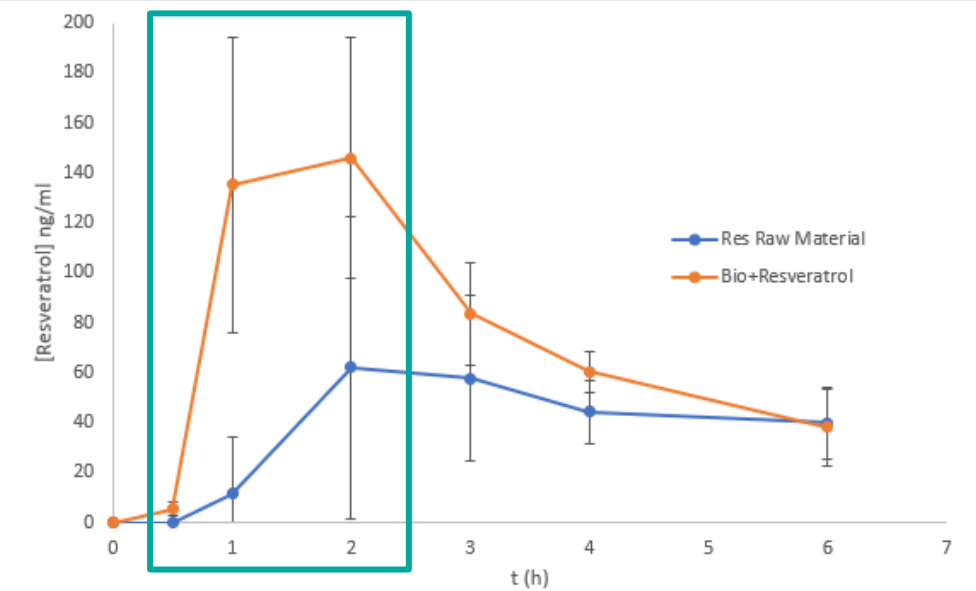
with



Resveratrol
from
Polygonum Cuspidatum
PCE07

Human Clinical Trial Results

Plasma - Resveratrol concentrations



The graphical result shows a clear trend towards a faster and higher peak concentration of resveratrol with Bio+Resveratrol®.
The first hours are the most important for gastric emptying and intestinal absorption.
In 1 hour an increase of 12 times of resveratrol concentration with Bio+Resveratrol® has been detected.

Pharmacokinetic calculations trend in human plasma confirmed resveratrol of BIO+Resveratrol® to have
HIGHER ASBORPTION and BIOAVAILABILITY
compared to pure resveratrol.
The efficacy of 125 mg of Resveratrol in BIO+Resveratrol® could be compared to 500 mg of standard one

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INNOVATION

BIO+Resveratrol®
Capsules

NUTRAE
by LAYN

bio+Resveratrol

ENHANCED BIOAVAILABILITY

in final nutraceutical form

CAPSULES



- **125 mg of Resveratrol per capsule**

- **417 mg of Bio+Resveratrol® per capsule (70% technology – 30% (125mg) Resveratrol)**

- Amount: 590 mg
  (BIO+Resveratrol® and Cellulose excipients)

- Blister: ALU / PVC

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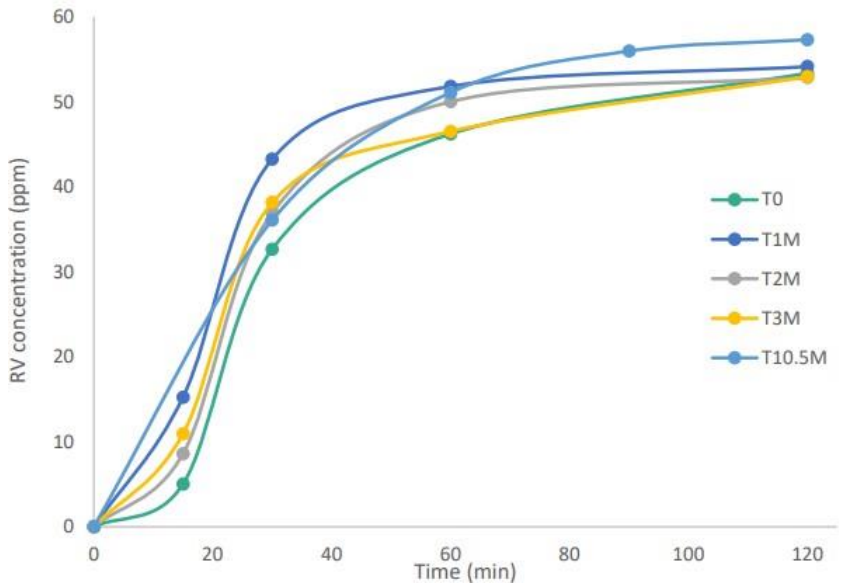
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BIO+Resveratrol®
Stability Study

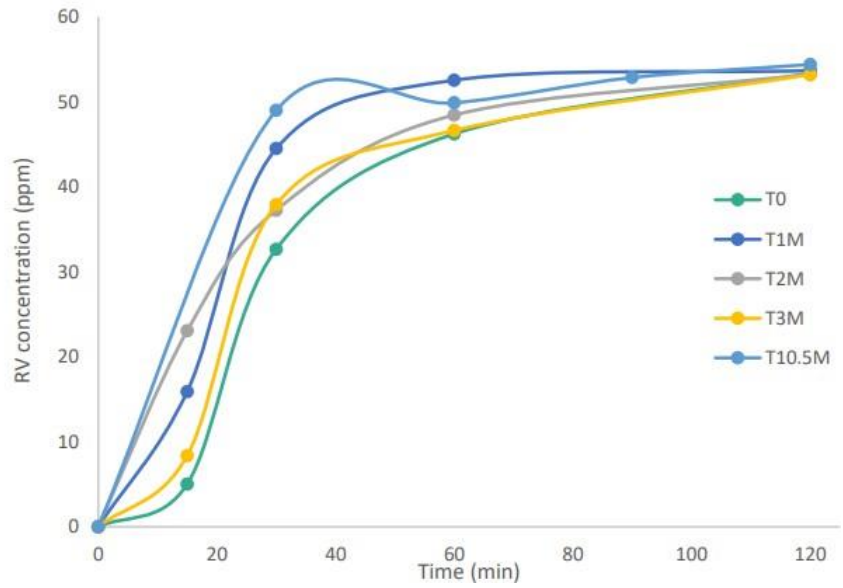
NUTRAE
by LAYN

Stability trial of Resveratrol in capsule

Ageing condition: RT



Ageing condition: 30°C/75%RH



BIO+Resveratrol® dissolution properties are still stable after 10 months of ageing at 30°C/75% RH and RT.

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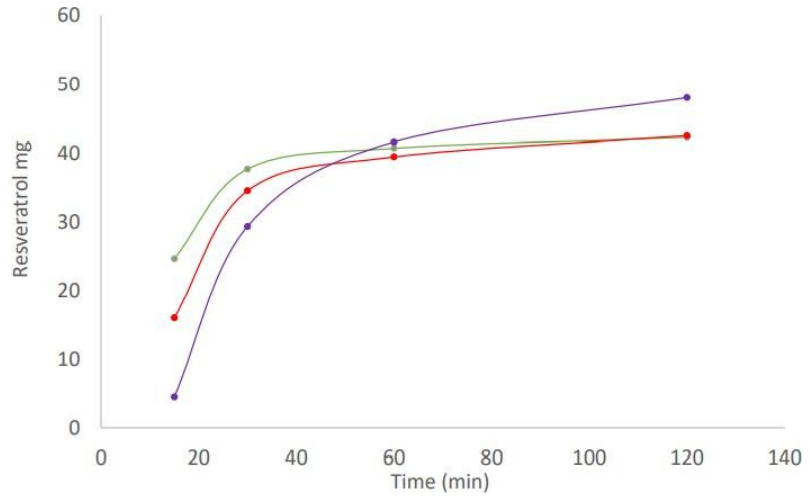
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INNOVATION

BIO+Resveratrol®
Competitors comparison

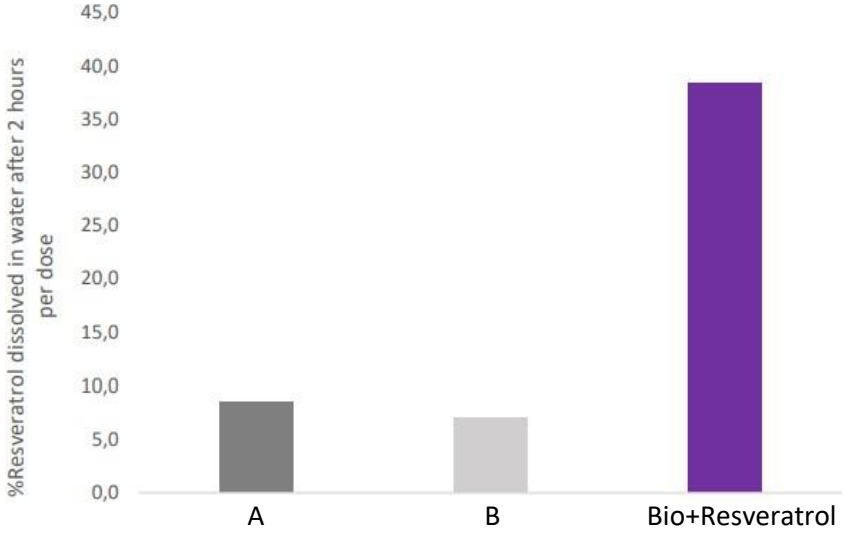
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Comparison of in-vitro release of BIO+Resveratrol® capsules with competitors



Graph:
Resveratrol dissolution kinetics for capsules with,
125 mg of resveratrol from BIO+Resveratrol® (**purple**),
500 mg of resveratrol from the market - competitor A (**green**),
600 mg of resveratrol from the market - competitor B (**red**).

- ✓ The resveratrol amount released by the competitors A and B is comparable to the one obtained by BIO+Resveratrol® (capsule version) between 30 minutes and the end of the trial (120 minutes).
- ✓ The significant differences observed between A, B and BIO+Resveratrol® after 15 minutes of trial are related to the dissolution time of the different capsules used for BIO+Resveratrol® and the competitors.
- ✓ <u>The amount of resveratrol dissolved for 125mg of this active in BIO+Resveratrol® is comparable to that of A and B with respectively 4 or 4.8 times less resveratrol compared to these competitors' products.</u>



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Thanks for your time!

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