



A novel, liquid trans-resveratrol containing nutraceutical preparation possessing SIRT1 gene activating properties as an option for consumer trans-resveratrol supplementation

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ABSTRACT:

Trans-resveratrol has been recognized as a substance derived from red grapes possessing Sirtuin gene activating properties. The Sirtuin gene family catalyzes various NAD(+)-dependent protein deacetylation reactions which result in critical regulation of aging, including transcriptive, apoptotic, and metabolic processes. While seven human sirtuins exist (SIRT1-7), SIRT1 has been implicated as a key mediator of cellular pathways associated with delaying the onset of age-related diseases and to potentially reduce morbidity associated with disease processes already in place. With these promising results, there has been consumer emphasis on the utilization of trans-resveratrol supplements for the promotion of human health. Unfortunately, even with the implementation of US CFR21, the availability of nutraceutical grade trans-resveratrol supplements has been scarce and studies have clearly shown concern over the quality of various purported trans-resveratrol consumer products. In this study, we evaluated a novel liquid preparation (*Eniva ResVante*®) as a nutraceutical option for dietary supplementation of trans-resveratrol. Areas evaluated include verification of the presence of trans-resveratrol, analysis of heavy metals and microbiologic contamination, dissolution evaluation, whether the inclusion of other known stimulators of SIRT1 were included in the formulation or extracts from sources known to possess trans-resveratrol (such as red grapes), and enzymatic testing for SIRT1 gene activation properties. The preparation tested contained a physiologically meaningful quantity of trans-resveratrol per serving (100 mg) that was verified by HPLC, passed heavy metal and microbiologic testing (USP) specifications, possessed additional phytonutrient substances (1,000 mg complex of quercetin, grape seed extract, red wine and fruit extracts, cranberry extract, lycium berry extract, acai berry extract, pomegranate fruit extract and ferulic acid), met dissolution standards (USP) and demonstrated contents possessing enzymatic SIRT1 gene activation properties through biochemical fluorophore assaying. In conclusion, the liquid preparation tested (*Eniva ResVante*®) represents a viable option for consumers seeking a nutraceutical preparation of trans-resveratrol possessing SIRT 1 gene activating content properties in context of whole food extracts from grapes.

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SUMMARY TESTING METHODS AND RESULTS

Table 1. HPLC verification* of physiologically meaningful trans-resveratrol content

Potency			
Label Claim	Test	Test Method	ResVante Test Result
100 mg / serving	Trans-resveratrol	HPLC Method - JC vol.1085*	121 mg

* Vian, MA., Tomao, V., Gallet, S., Coulomb, PO., Lacombe, JM., Simple and rapid method for cis and trans resveratrol and piceid isomers determination in wine by high performance liquid chromatography (HPLC) using chromolith columns. Journal of Chromatography A,1085; 2005, 224-229.

Table 2. Heavy Metal, Microbiologic, Sulfites and Alcohol Testing Data

Purity				
Category	Test	Test Method	Specification	Result
Microbial				
	Total Aerobic Count	USP<2021>/TM # MICRO-4000	<1 x 10 ² CFU/mL	PASSED
	Yeast & Mold	USP<2021>/TM # MICRO-4001	<1 x 10 ² CFU/mL	PASSED
	Salmonella	USP<2022>/TM # MICRO-4002	None Isolated	PASSED
	Coliforms	USP<2022>/TM # MICRO-4003	<1 x 10 CFU/mL	PASSED
	E. Coli	USP<2022>/TM # MICRO-4004	None Isolated	PASSED
	S. Aureus	USP<2022>/TM # MICRO-4005	None Isolated	PASSED
Heavy Metals				
	Mercury	ICP/MS/ TM # ICP-2000	< 6.76 mcg/serv	PASSED
	Arsenic	ICP/MS/ TM # ICP-2000	< 3.26 mcg/serv	PASSED
	Cadmium	ICP/MS/ TM # ICP-2000	< 2.10 mcg/serv	PASSED
	Lead	ICP/MS/ TM # ICP-2000	< 6.76 mcg/serv	PASSED
Other				
	Sulfites	AOAC 990.28	< 10 ppm	PASSED
	Alcohol content	AOCS BA13-87/ USP	< 0.5%	PASSED

Table 3. Dissolution Testing Data

Dissolution				
Category	Test	Test Method	Specification	Result
Pharmacokinetics	Dissolution standard for absorption	USP 24 <711> ***	60 seconds to 12 hours	100% Dissolution within less than 60 seconds

**Analysis performed by USP 24 <711> dissolution method in neutral buffered saline (1mM) @ 37°C with constant agitation in 40 mesh stainless steel baskets. End point determination of 12 hr. used to determine maximal solubility for residual solid calculation.

Table 4. Additional Nutrient Content

Phytonutrient Additions		
Nutrients and extracts	Quantity / serving	Comment
Red wine and fruit extracts, grape seed extract (GSE), quercetin, cranberry extract, lycium berry extract, acai berry extract, pomegranate fruit extract, ferulic acid.	1,000 mg / serving	Trans-resveratrol occurs in nature within the context of other phytonutrients found in various fruits, such as grapes. Design of nutritional products may seek to incorporate these cofactors through extract inclusion. The addition of naturally occurring antioxidants for nutrient stabilization may also be included for structural integrity and stability.

Enzymatic SIRT1 Gene Activation Testing

DESCRIPTION OF ASSAY USED:

The method used for SIRT1 activating properties is based on a Fluorescent Activity Assay/Drug Discovery system designed to measure the lysyl deacetylase activity of recombinant human SIRT1.

The *SIRT1 Fluorescent Activity Assay* is based on the unique Fluor de Lys[®]-SIRT1 Substrate/Developer II combination (see figure 1). The Fluor de Lys[®]-SIRT1 Substrate is a peptide comprising amino acids 379-382 of human p53 (Arg-His-Lys-Lys(Ac)). The assay's fluorescence signal is generated in proportion to the amount of deacetylation of the lysine corresponding to Lys-382, a known *in vivo* target of SIRT1 activity⁸⁻¹⁰. Fluor de Lys[®]-SIRT1 was the substrate deacetylated most efficiently by SIRT1 from among a panel of substrates patterned on p53, histone H3 and histone H4 acetylation sites (see Fig. 2, Fluor de Lys[®]-SIRT1 is labeled 'p53-382').

The assay procedure has two steps (Fig. 1). First, the Fluor de Lys[®]-SIRT1 Substrate, which comprises the p53 sequence ArBML-GHis-Lys-Lys(-acetyl), is incubated with human recombinant SIRT1 together with the cosubstrate NAD⁺. Deacetylation of Fluor de Lys[®]-SIRT1 sensitizes it so that, in the second step, treatment with the Fluor de Lys[®] Developer II produces a fluorophore.

Figure 1. Reaction Scheme of the SIRT1 Fluorescent Activity Assay

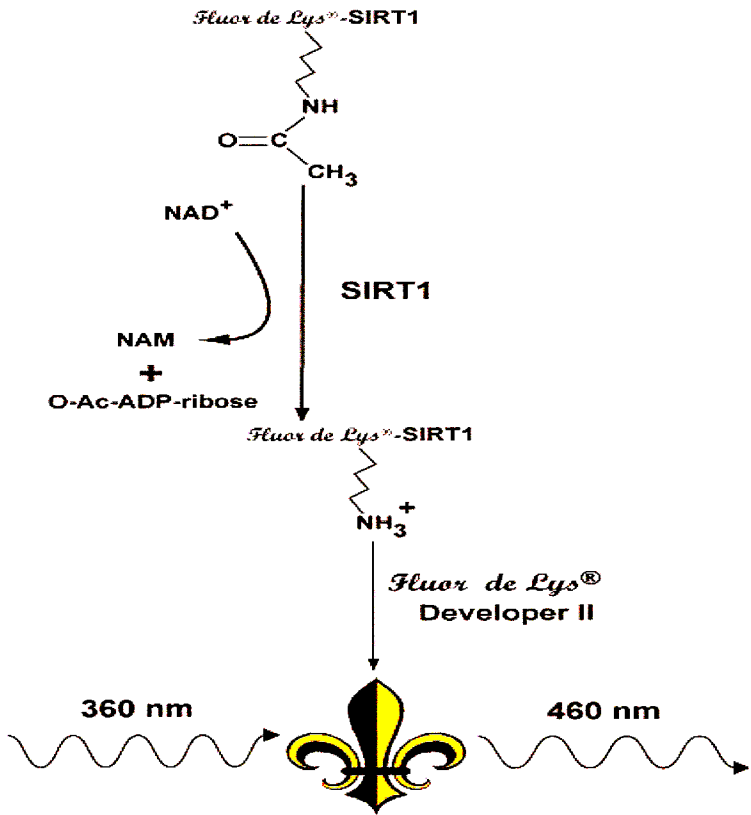


Figure 1. Reaction Scheme of the SIRT1 Fluorescent Activity Assay

NAD⁺-dependent deacetylation of the substrate by recombinant human SIRT1 sensitizes it to Developer II, which then generates a fluorophore (symbol). The fluorophore is excited with 360 nm light and the emitted light (460 nm) is detected on a fluorometric plate reader. NAD⁺ is consumed in the reaction to produce nicotinamide (NAM) and O-acetyl-ADP-ribose.

Figure 2. SIRT1 Peptide Substrate Preferences

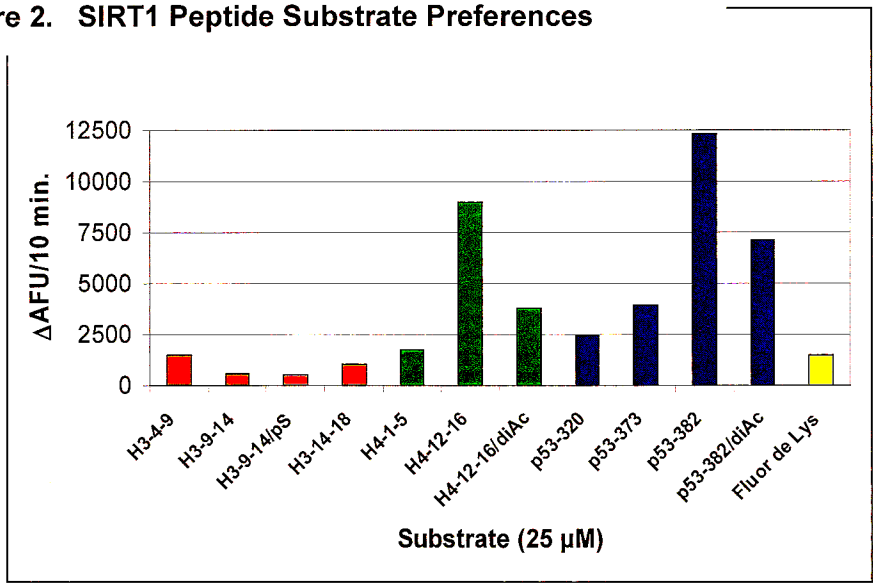


Figure 2. SIRT1 Peptide Substrate Preferences.

Initial rates of deacetylation were determined for a series of fluorogenic acetylated peptide substrates based on short stretches of human histone H3, H4 and p53 sequence. Recombinant human SIRT1 (1 U, BML-SE239), was incubated for 10 min at 37°C with 25 μM of the indicated fluorogenic acetylated peptide substrate and 500 μM NAD⁺. Reactions were stopped by the addition of Developer II/2 mM nicotinamide and the deacetylation-dependent fluorescent signal was allowed to develop for 45 min. Fluorescence was then measured in the wells of a clear microplate (BML-KI101) with a CytoFluor™ II fluorescence plate reader (PerSeptive Biosystems, Ex. 360 nm, Em. 460 nm, gain=85).

ENIVA TESTING RESULTS

Testing of the Eniva ResVante trans-resveratrol by the above described assay demonstrated clear SIRT1 activation properties. Both individually and in context of the full ResVante Red Wine Complex, SIRT1 activation was present.

Table 5. Change in arbitrary fluorescence units of Negative Controls vs Resvante trans-Resveratrol, triplicate data.

Sample	Δ AFU/30 min	Δ AFU/30 min	Δ AFU/30 min
Negative Control 1: Control solution with 0.1% v/v DMSO	406	400	423
Eniva ResVante trans-resveratrol	3,612	3,677	3,491
Negative Control 2: Control solution without DMSO	466	449	423

Figure 3.

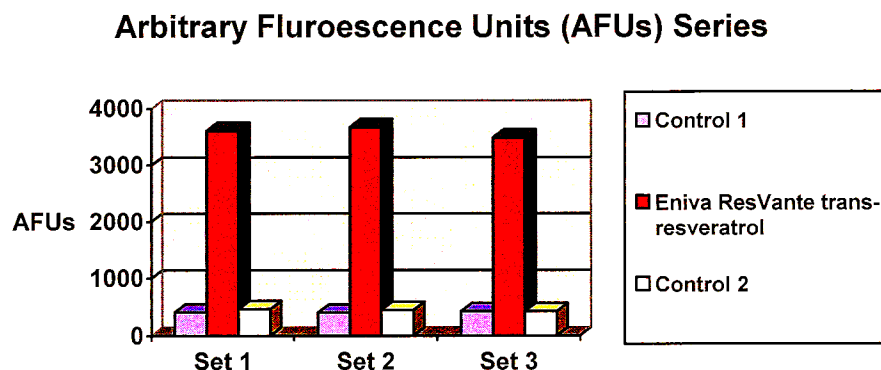


Figure 3. Change in arbitrary fluorescence units of Negative Controls versus Eniva ResVante trans-resveratrol, triplicate data.

Data points run in triplicate represent statistically significant changes in fluorescence value for the Eniva ResVante trans-resveratrol versus negative controls-- implicating SIRT1 activity.

Figure 4.

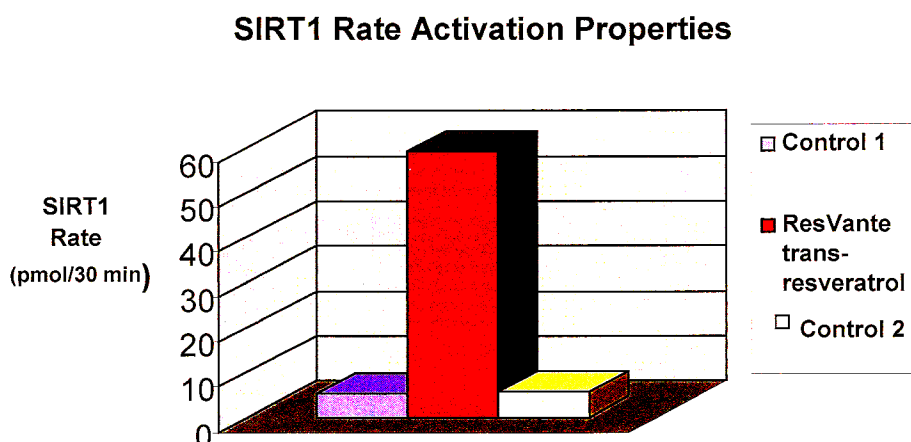


Figure 4. Results of SIRT1 Rate activation properties from standard curve calculation in relation to change in arbitrary fluorescence : Eniva ResVante trans-resveratrol

Data points clearly demonstrate statistically significant changes in the SIRT1 activation rate for the Eniva ResVante trans-resveratrol versus negative controls.

Discussion:

From recent studies, it is clear that trans-resveratrol holds great potential to support human health. While pharmaceutical development of trans-resveratrol containing drugs is underway, there is high interest in increasing dietary intake of trans-resveratrol through the use of dietary supplements. Unfortunately, even with the implementation of US CFR21 manufacturing standards, there remains significant concern regarding the quality and safety of trans-resveratrol supplements-- ranging from issues with contaminants, active ingredient integrity and pharmacokinetic design; let alone demonstrated gene activation properties. As well, an increasing subset of consumers are seeking supplements with extracts from whole food sources related to the nutrient of interest in an effort to capture naturally occurring complementary phytonutrients that may be as of yet undiscovered or unappreciated.

The current study of the Eniva ResVante nutraceutical provides insight into various quality aspects of this currently available trans-resveratrol dietary supplement. The Eniva ResVante nutraceutical was found to have satisfactory testing in all areas of evaluation, including HPLC verification of the presence of trans-resveratrol at the label claimed amount, safety analysis of heavy metals and microbiologic contamination, dissolution evaluation, and enzymatic testing demonstrating SIRT1 gene activation properties of its contents. In addition, the product was also found to have the presence of additional substances known to promote SIRT1 activity, such as quercetin, and the presence of extracts from sources known to possess naturally occurring trans-resveratrol, such as red grapes.

Conclusion:

The liquid preparation tested (*Eniva ResVante*®) represents a viable option for consumers seeking a liquid nutraceutical preparation of verified trans-resveratrol possessing SIRT 1 gene activating content properties in the context of whole food extracts from grapes.

References:

See references above and final publication manuscript.