BioActives LLC

1 Dix Street
Worcester, MA 01609
508-752-1099
508-752-0993 fax

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MicroActive® Resveratrol Powder (30%) *In Vitro* and *In Vivo* Studies

MicroActive® Resveratrol Dissolution Test

The objectives of the study were to determine the *in vitro* dissolution profile and sustained release properties of the MicroActive® Resveratrol formulation in comparison with Resveratrol powder. The studies were done using a biorelevant media, which can predict the bioavailability *in vivo.*

Microactive® Resveratrol Formulation

Resveratrol was formulated using a combination of HPMC, β-cyclodextrin and carnauba wax. The formulation is a powder containing 30% Resveratrol, suitable for hard gel capsules.

Samples Tested

Resveratrol powder (98%)

MicroActive® Resveratrol powder (30%)

Dissolution Test

The *in vitro* dissolution study was performed using the Varian 7020 dissolution tester with the basket configuration at 37^{o}C, 100rpm. The samples equivalent to 25mg Resveratrol filled in hard gelatin capsules were used for the study. The capsules were introduced into 750ml of 0.1N HCl (simulated gastric fluid without enzymes, SGF) maintained at 37^{o}C. At the end of 1 and 2 hrs, 3ml of the sample was withdrawn and filtered through 10μ syringe filter. The removed volume was replaced each time with fresh medium. At the end of 2 hr, the pH of the medium was adjusted to 6.5 (Simulated intestinal fluid without enzymes, SIF) with 195ml of 0.2M tribasic sodium phosphate

solution equilibrated to 37°C. Tween 80 dissolved in 55 ml of water was added to a concentration of 0.25% to simulate intestinal fluid. Aliquots were withdrawn at 4,6,9 and 12 hrs for analysis as described before and the removed volume was replaced with fresh medium each time. The aliquots were diluted with methanol for HPLC analysis. Resveratrol was quantified using reverse-phase HPLC on a Phenomenex Prodigy column (250x4.6mm, 5μm) at room temperature. The samples were eluted using an isocratic mobile phase consisting of 5% acetic acid/55% and methaniol/45%. The flow rate was 1ml/min and detection wavelength was 306nm. The retention time for Resveratrol was 7.5 min under these conditions. Standard Resveratrol from Sigma was used for calibration.

Observations

Since Resveratrol has low solubility in water, the dissolution study was done under sink conditions to allow enough media for complete dissolution. Figure 1 presents the dissolution profile of Resveratrol powder and MicroActive® Resveratrol. The Resveratrol powder showed complete dissolution (100%) in 4 hours. The MicroActive® formula showed sustained release over a period of 12hrs, with 97% of the dose released at 12 hrs.

MicroActive® Resveratrol Bioavailability: A Pilot Study

The objective of this human study was to determine the uptake of MicroActive® Resveratrol powder in comparison with 98% Resveratrol powder.

Clinical Study Protocol

MicroActive® Resveratrol formulation and 98% Resveratrol powder equivalent to 500mg Resveratrol were used for the study. The samples were provided in hard gelatin capsules. The cross-over study was done using two healthy subjects who were not consuming Resveratrol supplements. The subjects were asked not to consume Resveratrol containing foods such as peanuts grapes and wine for 48 hours before the study. On the day of the study, 7ml of blood was collected in EDTA tubes to permit establishment of

baseline value. MicroActive® Resveratrol capsules were administered after breakfast and blood samples were drawn as before at 1,2,4,7, 9, and 24 hrs post-dose. The samples were stored on ice protected from light and the plasma was separated by centrifugation within 1hr of collection and stored at -70°C till analysis. After a 7-day washout period the protocol was repeated for the control Resveratrol powder capsules.

Resveratrol Extraction and Analysis

Plasma total Resveratrol (Resveratrol conjugates and free Resveratrol) and free Resveratrol were determined based on the method described by Meng et al (Urinary and plasm levels of resveratrol and quercetin in humans, mice and rats after ingestion of pure compounds and grape juice, J. Agric. Food Chem., 52: 935-942, 2004). For total Resveratrol analysis, 0.5 ml of the plasma was incubated with 1000 units of β-glucuronidase and 130 units sulfatase at 37°C for 1 hr. The proteins were precipitated by adding 0.5 ml methanol to the plasma samples. The mixture was extracted twice with 2.5ml ethyl acetate and the solvent was evaporated under nitrogen protected from light. The residue was dissolved in the mobile phase 5% acetic acid/55% and methanol/45% (0.2ml) for HPLC analysis. For free Resveratrol analysis, the samples were extracted as described without enzyme hydrolysis. Resveratrol was quantified using reverse-phase HPLC as described above.

Results and Observations

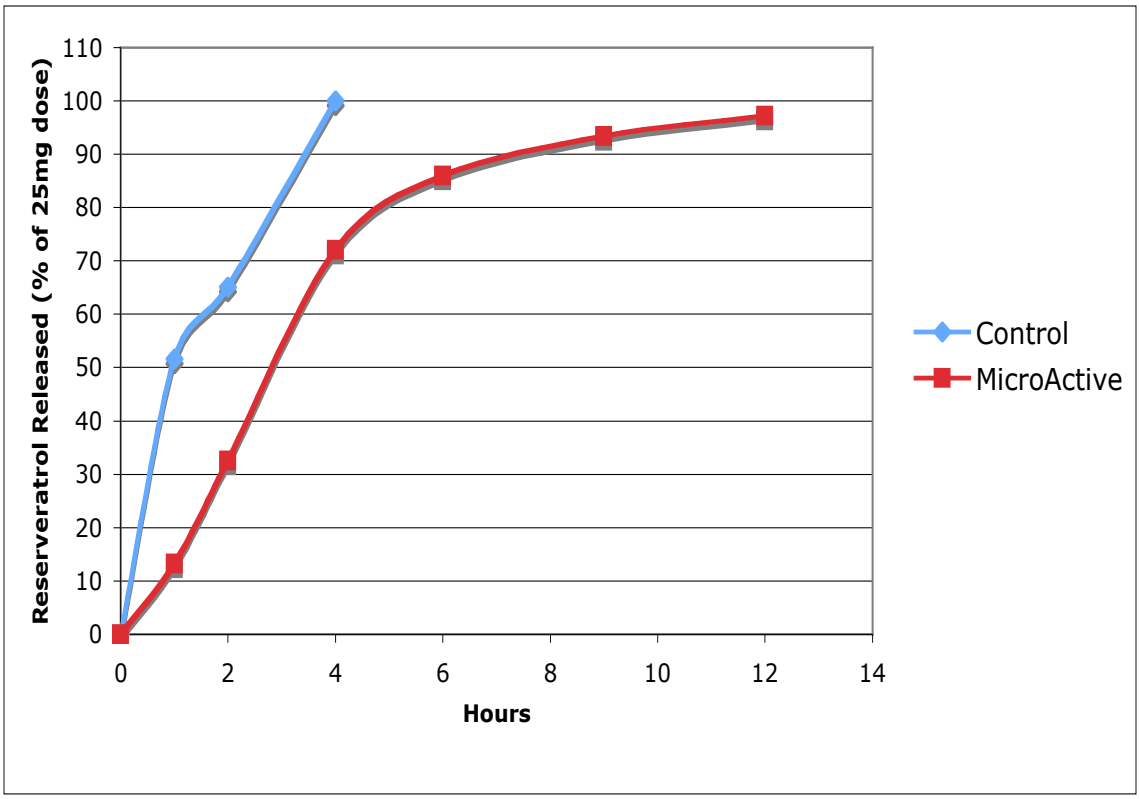
Figure 2 presents the plasma total Resveratrol profile from MicroActive® Resveratrol and 98% Resveratrol powder. Resveratrol powder showed a rapid absorption with a sharp peak at T_{max} of 2 hrs post-dose. After 2 hrs, the plasma levels of total Resveratrol declined rapidly with a small increase at 9 hrs. MicroActive® Resveratrol also showed a T_{max} at 2 hr, but a lower C_{max} (maximum concentration at T_{max}) with a broader peak and higher plasma levels compared to the control for >9 hrs.

The free Resveratrol profile (Figure 3) indicates higher plasma levels with MicroActive Resveratrol, which was maintained for >9 hrs compared to the control. The average area under the curve ($AUC_{0\text{-}24hr\ mcg/(ml*hr)}$) for free Resveratrol was 5.84 (MicroActive®

Resveratrol) and 2.29 (control). The ratio of MicroActive® to control Resveratrol AUC was 2.54.

Unformulated Resveratrol is absorbed rapidly, metabolized extensively and eliminated, resulting in trace amounts of unchanged Resveratrol in systemic circulation. The results of the study indicate that MicroActive® sustained-release formulation of Resveratrol extends the systemic exposure to higher levels of free Resveratrol and its metabolites. The results warrant a larger clinical study for further validation.

Figure 1. A Comparison of Dissolution Profile of Resveratrol



pH1.0 pH6.5

Figure 2. Total Resveratrol Profile *In Vivo*

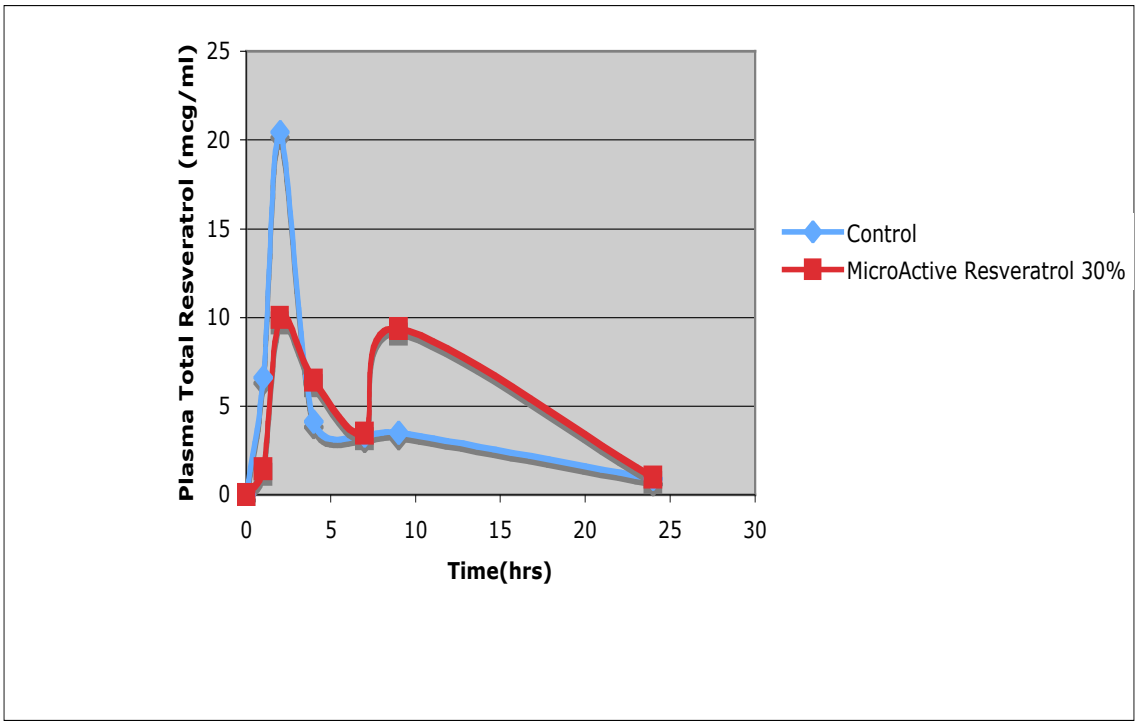


Figure 3. Free Resveratrol Profile *In Vivo*