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In vitro drug release and *ex vivo* percutaneous absorption of resveratrol cream using HPLC with zirconized silica stationary phase

Hudson Caetano Polonini[a,*], Carina de Almeida Bastos[b], Marcone Augusto Leal de Oliveira[b], Carla Grazieli Azevedo da Silva[c], Carol Hollingworth Collins[c], Marcos Antônio Fernandes Brandão[a], Nádia Rezende Barbosa Raposo[a]

[a] *Núcleo de Pesquisa e Inovação em Ciências da Saúde (NUPICS), Federal University of Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil*
[b] *Grupo de Química Analítica e Quimiometria (GQAQ), Federal University of Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil*
[c] *Institute of Chemistry, University of Campinas, PO Box 6154, 13083-970 Campinas, SP, Brazil*

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ABSTRACT

Since the designs of optimal formulations for resveratrol permeation *via* the skin are lacking, the aim of this study was to establish the profile of resveratrol permeability into and across human skin. For that, a laboratory-made chromatographic column was used (Zr-PMODS), with its performance being compared to a traditional C18 column. *In vitro* drug release was conducted with polysulfone membranes, and the flux (J_S) was 30.49 μg cm^{-2} h^{-1}), with a lag time (L_T) of 0.04 h, following a pseudo-first-order kinetics. For *ex vivo* percutaneous absorption using excised female human skin, the kinetic profile was the same, but J_S was 0.87 μg cm^{-2} h^{-1} and L_T was 0.97 h. From the initials 49.30 μg applied to the skin, 9.50 μg were quantified in the receptor medium, 20.48 μg was retained at the *stratum corneum* (do not account as permeated) and 21.41 μg was retained at the viable epidermis + dermis (account as permeated), totalizing 30.90 μg of resveratrol permeated after 24 h of application (62.6%). From these results, one can conclude that a person using the 1-g emulsion dose released by the pump containing 20 mg of resveratrol will have, theoretically, 12.53 mg of it liberated into his bloodstream, gradually and continuously for 24 h.

1. Introduction

Resveratrol (3,5,4-trihydroxystilbene) (Fig. 1) is a naturally occurring non-flavanoid phenolic compound produced by some spermatophytes, such as grapes [1]. It is found in high concentrations in many red grape skins and their wines [2], with the *trans*-resveratrol isomer being more biologically active [3].

The substance has been reported to possess antioxidant, neuroprotective, antiphotoaging and antiviral activities, and it seems that it also plays a role in the prevention and reduction of pathological processes such as inflammation, cancer and heart diseases [4]. However, it has poor oral bioavailability, which creates a dilemma between its great *in vitro* efficacy and its low *in vivo* effect [5], mainly because it is extensively metabolized in the body [6].

On the other hand, topically applied resveratrol has been shown to possess more pronounced properties, namely: it is antiproliferative and chemopreventive against skin carcinogenesis [7]; gives sunprotection against skin damage from ultraviolet B (UVB) exposure [8]; is antimicrobial against dermatophytes and herpes simplex virus [1,9]; and activates estrogen receptors [10]. Thus, skin delivery of resveratrol is paramount for the effective insertion of the substance into pharmaceuticals, either topical or transdermal.

According to literature reports, the stationary phase most commonly used for resveratrol analysis is the octadecyl-bonded silica (C18), whether in nutraceuticals [11], grape fruits [12], wines [13] or biological samples [14]. However, to the best of the authors' knowledge transdermal emulsions containing resveratrol for human use have not been investigated so far, although one previous study have reported encouraging data for solutions and hydrogels [15].

Since the designs of optimal formulations for resveratrol permeation *via* the skin are lacking [15], the aim of this study was to establish the profile of resveratrol permeability into and across human skin. For that, a laboratory-made chromatographic column was used (Zr-PMODS), with its performance being compared to a commercial C18 column.

* Corresponding author at: Federal University of Juiz de Fora, Rua José Lourenço Kelmer, s/n, Campus Universitário, 36036-900, Juiz de Fora, MG, Brazil. Tel.: +55 32 21023809; fax: +55 32 21023809.
E-mail addresses: h.c.polonini@gmail.com (H.C. Polonini), marcone.oliveira@ufjf.edu.br (M.A.L.d. Oliveira), marcosbrand2012@gmail.com (M.A.F. Brandão), nadiafox@gmail.com (N.R.B. Raposo).

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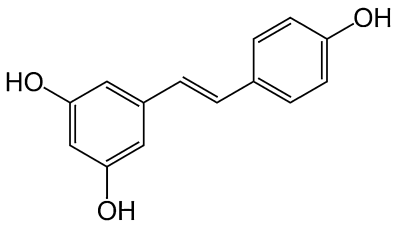

Fig. 1. Resveratrol chemical structure.

2. Materials and methods

2.1. Reagents, standard and materials

The acetonitrile (ACN) used in the preparation of the mobile phase was HPLC grade, and sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), and potassium dihydrogen phosphate (KH_2PO_4) were of analytical grade, all from Sigma-Aldrich (USA). Ultrapure water (H_2O) obtained in an AquaMax-*Ultra* 370 Series (Young Lin, Korea) (18.2 MΩ cm resistivity at 25 °C and <10 ppb total organic carbon) was used throughout analysis. Resveratrol 98% was from JiAherb (China), and ethoxydiglycol and Pentravan® for emulsions compounding were kindly donated by Fagron (Brazil). The reference standard used was also from JiAherb. All the mobile phases and receptor media were filtered through a 0.45-μm filter membrane (Sartorius, Germany) under vacuum and degassed by an ultrasonic apparatus (Cristófoli, Brazil) for 30 min before use. All volumetric glassware was previously calibrated.

2.2. Transdermal emulsion

The composition (m/m) of the transdermal product was: resveratrol, 2%; ethoxidiglycol, 0.5%; and vehicle in quantity sufficient for 1 g. The resveratrol was accurately weighed, transferred to an agate mortar, ground stepwise with the ethoxydiglycol and then geometrically homogenized with the Pentravan® vehicle. The product was passed through a roll mill (Fagron, USA), collected, and packed into white airless plunger packing (Emphasys, Brazil).

2.3. Standard and sample solutions

Accurately weighed amounts (analytical digital balance AY220, Shimadzu, Japan) of the standard were dissolved and diluted in receptor medium to obtain working solutions. The transdermal emulsion was diluted in the same manner, but using an ultrasonic bath (10 min) to improve solubilization.

2.4. Quantification of resveratrol by HPLC—Method optimization

The HPLC analyses were performed in a qualified and calibrated Young Lin (Korea) chromatography system composed by: quaternary pump (YL 9110), photodiode array detector (YL 9160), automatic injector (YL 9150), column compartment (YL 9130) and software controller (Clarity). Chromatographic separation was achieved using a C18, 250 × 4.6 mm, 5 μm particle size, commercial column (Phenomenex, USA) or a column containing poly(methyloctadecylsiloxane) thermally immobilized onto zirconized silica, 250 × 4.6 mm, 5 μm particle size, as described by da Silva and Collins [16,17].

In order to minimize time and reagent and optimize the factors of the chromatographic system, an experimental design approach was performed. The following parameters were evaluated: acetonitrile percentage in the mobile phase (composed solely of acetonitrile and water), temperature of the column oven, and the identity of the column itself (commercial or laboratory-made). To evaluate the significance that each parameter/factor had on the assay of resveratrol and to determine the optimal conditions for inclusion of the method in routine laboratorial tests, a $3^2 \times 2$ factorial design with mixed levels containing three factor levels (−1, 0, +1 for the factors ACN percentage and column oven temperature) and two (−1 and +1 for the factor column) was randomly conducted in a total of 18 experiments with three replicates in each experimental level. The factors and their levels are listed in Table 1. After the experiments, statistical analysis was conducted accordingly to Polonini et al. [18]. Other experimental conditions such as injection loop of 20-μL; mobile phase at a flow rate of 1.2 mL min^{-1}; and wavelength of detection at 307 nm were maintained constants.

2.5. Validation

After method development and optimization, the validation tests were performed according to the International Conference on Harmonization (ICH) [19] and the Brazilian National Institute of Metrology, Standardization and Industrial Quality (INMETRO) [20] guidelines, comprising the following parameters:

2.5.1. Specificity

The specificity was determined by a Student's *t*-test for comparison of the resveratrol quantification (mean values) from the analyte with or without the matrix (Pentravan® + ethoxydiglycol). The acceptance criterion was defined as the percentage of discrepancy between these results being lower than 2%. Additionally, the specificity of the method was obtained through the comparison of the chromatograms of the standards with and without the matrix.

2.5.2. Linearity

The test was conducted by the plotting of three standard curves, each constructed from the initial resveratrol concentrations of 4, 10, 20, 40, 60, 80 and 160 μg mL^{-1} (added with the concentrations of 0.15, 0.50, 1.0 and 2.0 μg mL^{-1}, after the theoretical determination of the limit of quantification) in order to assess the linear relationship between the concentration of the analyte and the obtained areas. For this purpose, the data for each concentration range of the final curve (0.15–160 μg mL^{-1}) after fitting by ordinary least squares method were statistically evaluated taking into account homoscedasticity (Cochran's test), residues' normality (Shapiro–Wilk's test) and lack of fit test (ANOVA).

2.5.3. Limits of detection and quantification

The theoretical limits of detection (LOD) and quantification (LOQ) were determined from three standard calibration curves and were calculated as shown in Eqs. (1) and (2), respectively:

$$\mathrm{LOD} = S\frac{3}{a} \quad (1)$$

$$\mathrm{LOQ} = S\frac{10}{a} \quad (2)$$

where *a* is the slope of the calibration curve and *S* is the standard deviation of the *y*-intercept.

The practical LOD and LOQ were confirmed by analysis of the chromatogram generated by injecting solutions in their respective limit concentrations, and then the average standard curve was added with these concentrations, to provide quantification in the low concentrations demanded for the permeation studies.

2.5.4. Precision

The test was designed to assess the degree of dispersion between the series of measurements obtained by the same analyst (intra-assay precision, repeatability) and between two analysts and two