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

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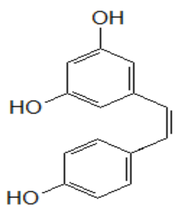
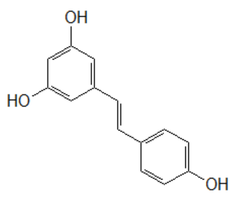
INTRODUCTION



cis-resveratrol



trans-resveratrol

- Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenol compound naturally occurring in high to moderate quantities in various foods including grapes, peanuts and wine. Chemically, it has two geometrical isomers, *trans*- and *cis*-resveratrol, been the *trans* form biologically more active and also more stable.

- Resveratrol has anti-tumor, anti-inflammatory, anti-oxidant, photoprotective ,depigmentation and anti-platelet aggregation properties.

- The combination of several limiting factors including poor water solubility, limited chemical stability, short biological half-life, and rapid metabolism and elimination means that resveratrol demonstrates low bioavailability, especially by oral route.

- Once resveratrol has physicochemical properties that are particularly suitable for diffusion through the human skin, notably its low molecular weight (228.25 g mol^{-1}) and its adequate lipophilicity (log $P_{o/w}$ = 3.1), a transdermal system could be an alternative drug delivery mechanism.

OBJECTIVE

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.

MATERIAL AND METHODS

Transdermal emulsion composition:
Trans-resveratrol 2%
Ethoxidiglycol 0.5%
Pentravan® cream, qs .....100%.

RP-HPLC

Method optimization
a 3^2× 2 factorial design with mixed levels containing three (−1, 0,+1 for the factors ACN percentage and column oven temperature) and two (−1 and +1 for the factor column) factor levels was randomly conducted in a total of 18 experiments with three replicates in each experimental level.

VALIDATION

Parameters
Robustness, specificity, linearity, limits of detection and quantification, precision and accuracy.

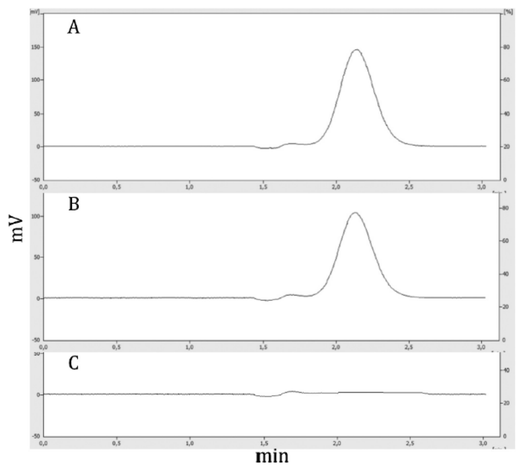
Issues	Contrast coefficients										Response	
	Mean	X_1	X_2	X_3	X_1^2	X_2^2	X_1X_2	X_1X_3	X_2X_3	$X_1X_2X_3$	Average	SD
1	1	−1	−1	−1	1	1	1	1	1	−1	5026.288	84.207
2	1	0	−1	−1	0	1	0	0	1	0	5544.994	26.758
3	1	1	−1	−1	1	1	−1	−1	1	1	5600.582	20.705
4	1	−1	0	−1	1	0	0	1	0	0	5286.010	76.626
5	1	0	0	−1	0	0	0	0	0	0	4651.320	349.343
6	1	1	0	−1	1	0	0	−1	0	0	5663.926	8.738
7	1	−1	1	−1	1	1	−1	1	−1	1	5527.990	16.786
8	1	0	1	−1	0	1	0	0	−1	0	5740.165	36.511
9	1	1	1	−1	1	1	1	−1	−1	−1	5678.967	32.468
10	1	−1	−1	1	1	1	1	−1	−1	1	5267.115	11.632
11	1	0	−1	1	0	1	0	0	−1	0	5901.353	92.928
12	1	1	−1	1	1	1	−1	1	−1	−1	5470.780	220.406
13	1	−1	0	1	1	0	0	−1	0	0	5479.850	67.973
14	1	0	0	1	0	0	0	0	0	0	5695.148	177.105
15	1	1	0	1	1	0	0	1	0	0	5421.821	63.754
16	1	−1	1	1	1	1	−1	−1	1	−1	5819.251	36.127
17	1	0	1	1	0	1	0	0	1	0	5831.631	37.582
18	1	1	1	1	1	1	1	1	1	1	5512.429	63.407

X_1: acetonitrile in mobile phase (%): (−1):45; (0):50; (+1):55.
X_2: column temperature (°C): (−1):25; (0):35; (+1): 45.
X_3: column: (−1): C18 commercial column; (+1): Zr-PMODS.

HUMAN SKIN PERMEATION

Experimental conditions:
- Receptor medium: Artificial human sweat with 20% of ethanol as solubiliser.
- The receptor medium was maintained at 32 ± 2ºC and mixed with a magnetic stirring bar (600 rpm);
- Aliquots (1 mL) were withdrawn at regular time intervals (0.5, 1, 2, 3, 4, 8, 12, 16, 20 and 24h), collected into HPLC vials and *trans*-resveratrol was quantified;
- Drug retention: micrometric cuts using cryostat microtome.

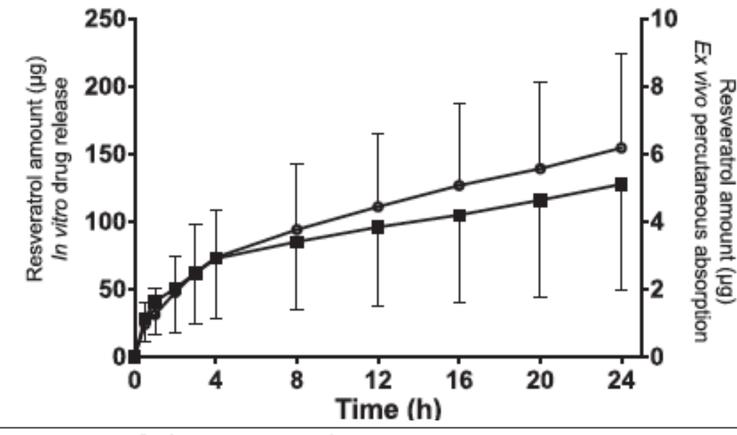
RESULTS



Zr-PMODS proved to be comparable to C18 commercial columns for HPLC quantification.
The figure shows chromatograms of (A) standard, (B) sample and (C) matrix. Chromatographical conditions set as: Zr-PMODS column (250 × 4.6 mm, 4.5 µm) at 25◦C; volume of injection of 20 µL; mobile phase consisted of acetonitrile : water (50:50, v/v) at a flow rate of 1.2 mL min^{-1}; and UV detection at 307 nm.

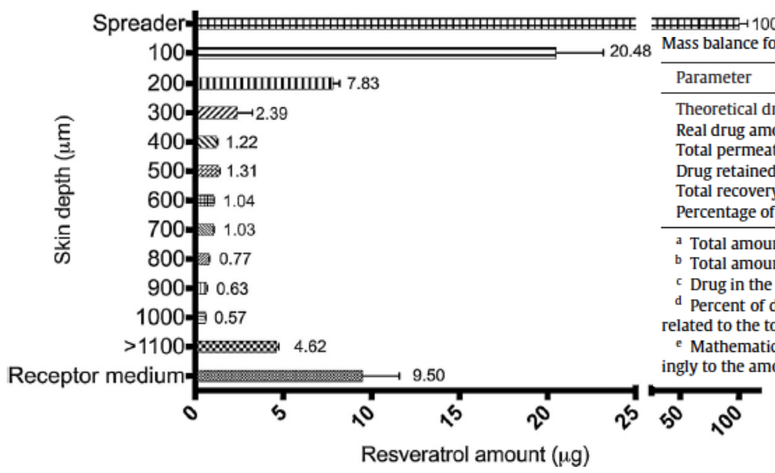
The method was validated and proved to be simple, selective, precise, accurate and fast and is in accordance to the validation parameters required by ICH guideline.

Cumulative amounts of resveratrol
quantified into the receptor medium: (O) in vitro drug release profile and (■) ex vivo percutaneous absorption profile (resultsp resented as mean ± standard deviation (n = 6).



Mathematical model	Equation	R^2	J_S (µg cm^{-2} h^{-1})	K_P (cm h^{-1})	T_L (h)	Cumulative drug in receptor media (µg)
Drug release						
Zero-order	$y = 5.22x + 39.26$	0.941				
Higuchi	$y = 30.49x + 5.67$	0.994	30.49	5.08	0.04	287.55
First-order	$y = 0.03x + 1.61$	0.783				
Percutaneous absorption						
Zero-order	$y = 0.15x + 1.79$	0.919				
Higuchi	$y = 0.87x + 0.82$	0.984	0.87	5.85	0.97	9.50
First-order	$y = 0.02x + 0.25$	0.781				

J_S = steady-state flux; K_p = permeability coefficient; T_L = lag time.
Results expressed as a mean of six replicates.



Mass balance for the transdermal permeation of resveratrol.

Parameter	Result
Theoretical drug amount applied to skin (µg)[a]	150.0
Real drug amount applied to skin (µg)[b]	49.3
Total permeated amount after 24 h (µg)[c]	30.9
Drug retained in *stratum corneum* (µg)	20.5
Total recovery (%)[d]	102.2
Percentage of permeation per dose (%)[e]	62.6

[a] Total amount of cream placed in the donor chamber (4.0 mg cm^{-2}).
[b] Total amount (*a*) minus the retained amount in the spreader.
[c] Drug in the receptor medium + viable epidermis + dermis.
[d] Percent of drug quantified in the spreader, the skin and the receptor medium, related to the total amount applied (*a*).
[e] Mathematical estimate of the total drug that would permeate the skin accordingly to the amount applied [(*c*/*b*) × 100].

Skin retention profile of resveratrol from liposomal transdermal emulsion. Results presented as mean ± standard deviation (n = 6).

CONCLUSION

Using the percentage of permeation by dose (62.6%), one can conclude that a person using the 1-g emulsion dose released b ythe pump containing 20 mg of resveratrol will have, theoretically, 12.53 mg of it liberated into his bloodstream, gradually and continuously for 24 h. To confirm that, the next step is to perform in vivo pharmacokinetic/pharmacodynamics studies.

For further information, please read:
Polonini et al. Journal of Chromatography B, 947-948 (2014) 23-31.
http://dx.doi.org/10.1016/j.jchromb.2013.12.005