

Supporting Information

Development and Validation of HPLC Method using a Monolithic Column for Quantification of trans-Resveratrol in Lipid Nanoparticles for Intestinal Permeability Studies

Ana Rute Neves, Salette Reis, and Marcela A. Segundo*

REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of
Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal.

* msegundo@ff.up.pt

Formula: C₁₄H₁₂O₃

Molecular weight: 228.24 g mol⁻¹

Water solubility, S_w: 30 mg L⁻¹

log P_{ow}: 3.40

pKa: 8.99, 9.63, 10.64

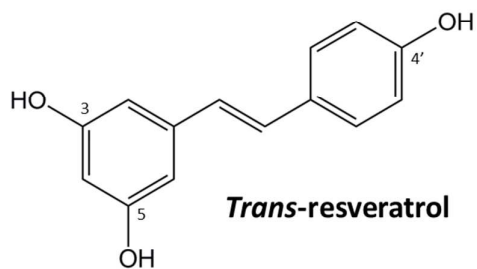


Figure S1 – Chemical structure and physicochemical properties of *trans*-resveratrol.

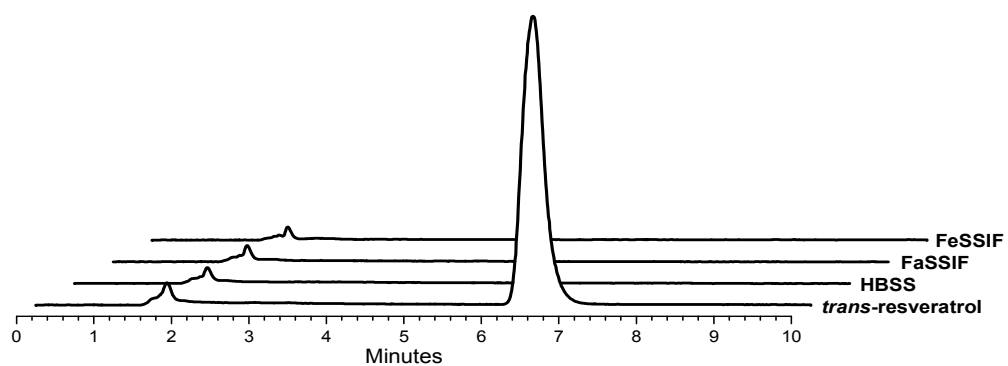


Figure S2 – Chromatograms of blank matrices HBSS, FaSSIF and FeSSIF and of *trans*-resveratrol ($10\ \mu\text{mol L}^{-1}$) in mobile phase containing acetic acid (2%, v/v):acetonitrile (80:20).

Table S1 – Summary of chromatographic conditions and figures of merit of HPLC methods for *trans*-resveratrol quantification.

Reference	Column	Mobile Phase	Elution Mode	Injection Volume (μl)	Flow rate (mL min ⁻¹)	Retention Time (min)	Detection	DL (μmol L ⁻¹)	QL (μmol L ⁻¹)	Linearity and Range (μmol L ⁻¹)	RSD (%)	Application
(5)	C18, 5 μm, 100 x 2.1 mm	(A) H ₂ O (B) MeOH	Gradient	5	0.5	7.3	DAD (306 nm; 286 nm)	1.2 (306 nm); 0.6 (286 nm)	n.a.	5-53	1.4 (306 nm); 1.7 (286 nm)	n.a.
(12)	C18, 5 μm, 150 x 4 mm	(A) ACN (B) 5% aqueous acetic acid	Gradient	50	1	17.2	DAD (300 nm); fluorescence (324/370 nm)	0.3 (DAD); 0.01 (fluo)	1 (DAD); 0.04 (fluo)	0.4-88 (DAD); 0.04-4 (fluo)	1.6 (DAD); 9.2 (fluo)	Wine
(13)	C18, 5 μm, 250 x 4.6 mm	H ₂ O:ACN (75:25)	Isocratic	20	1.5	8.0	UV-Vis (306 nm)	n.a.	n.a.	0.4-44	1.7 (intra-day); 3.2 (inter-day)	Wine
(14)	C18, 5 μm, 150 x 2.1 mm	(A) H ₂ O adjusted to pH 2.5 with sulfuric acid (B) ACN	Gradient	50	0.2	45.0	MS (m/z 228); DAD (305 nm)	5 (MS); 4 (DAD)	18 (MS); 14 (DAD)	11-220 (MS); 11-220 (DAD)	10.8 (MS); 2.8 (DAD)	Wine
(15)	C18, 5 μm, 250 x 2.0 mm	1% aqueous formic acid:ACN:2-propanol (70:22:8)	Isocratic	5	0.2	12.0	DAD (306 nm)	0.3	1.3	1.7-55	0.9-2.1 (intra-day); 1.3-2.8 (inter-day)	Wine, grapes
(16)	C18, 5 μm, 250 x 4 mm	25% ACN, 0.1% H ₃ PO ₄ and NaCl (5 mmol/L) in H ₂ O	Isocratic	20	1	n.a.	DAD (306 nm); electrochemical (0.75 V)	0.1 (DAD); 0.01 (0.75 V)	n.a.	0.04-40	2.3	Wine
(17)	C18, 5 μm, 250 x 4.6 mm	H ₂ O:ACN:Acetic acid (70:29.9:0.1)	Isocratic	20	1	10.5	DAD (310 nm)	0.3	n.a.	0.4-4	16.3-27.2	Wine
(18)	C18, 4 μm, 150 x 4.0 mm	ACN:phosphate buffer pH 4.8 (30 mM) (25:75)	Isocratic	20	1	4.6	DAD (310 nm)	0.4	1	0.6-18	0.9 (intra-day); 2.2 (inter-day)	Plasma
(32)	C18, 5 μm, 150 x 4.6 mm	(A) MeOH: acetic acid:H ₂ O (10:3:66) (B) MeOH: acetic acid:H ₂ O (90:2:8)	Gradient	20	1	11.0	fluorescence (330/374 nm)	0.09	0.3	0-65	2.6 (intra-day); 4.0 (inter-day)	Vine leaves
(19)	C18, 5 μm, 250 x 4.6 mm	ACN:H ₂ O (40:60) plus 0.1% TFA	Isocratic	10	1	4.7	DAD (308 nm)	n.a.	n.a.	0.04-26	n.a.	Peanuts, pistachios
(20)	C18, 6 μm, 250 x 4.6 mm	(A) MeOH:H ₂ O: acetic acid (10:90:1) (B) MeOH:H ₂ O: acetic acid (90:10:1)	Gradient	20	1.5	21.6	DAD (306 nm); MS (m/z 228)	0.05 (DAD); 0.02 (MS)	n.a.	0.04-440	n.a.	Wine
(21)	Chromolith RP-18e, 100 x 4.6 mm	(A) H ₂ O:acetic acid (94:6) (B) H ₂ O:ACN:acetic acid (65:30:5)	Gradient	20	4	10	DAD (306 nm)	0.1	0.4	0.4-4	n.a.	Wine
(22)	C18, 5 μm, 250 x 4.6	0.5% acetic acid in MeOH:H ₂ O (50:50)	Isocratic	25	1	4.3	DAD (303 nm)	n.a.	0.3	0.3-29	0.8-7.0 (intra-day); 2.4-7.0 (inter-day)	Plasma
(33)	C18, 3 μm, 100 x 3.2 mm	(A) H ₂ O:acetic acid (95:5) (B) ACN	Gradient	0.5	0.004	29.0	fluorescence (300/381 nm)	0.02	0.06	0.06-9	5	Wine
(23)	C18, 5 μm, 250 x 4.6 mm	(A) ACN (B) 0.5% aqueous acetic acid	Gradient	10	1	16.4	DAD (320 nm)	0.1	0.2	0.5-102	2.3-3.9 (intra-day); 3.3-4.2 (inter-day)	Plasma
(24)	C18, 5 μm, 250 x 4.6 mm	H ₂ O:ACN (70:30)	Isocratic	20	1	7.8	DAD (306 nm)	1	4	12-46	1.0 (intra-day); 1.8 (inter-day)	Dietary supplement _s

Table S1 – Summary of chromatographic conditions and figures of merit of HPLC methods for *trans*-resveratrol quantification (continuation).

Reference	Column	Mobile Phase	Elution Mode	Injection Volume (μl)	Flow rate (mL min ⁻¹)	Retention Time (min)	Detection	DL (μmol L ⁻¹)	QL (μmol L ⁻¹)	Linearity and Range (μmol L ⁻¹)	RSD (%)	Application
(35)	C18, 3.5 μm, 100 x 3.0 mm	1 mM ammonium acetate:ACN (73:27)	Isocratic	5	1	1.5	MS (m/z 227)	n.a.	n.a.	0.04-4	n.a.	Wine
(25)	C18, 4 μm, 250 x 4.6 mm	(A) 3% aqueous acetic acid (B) 3% aqueous acetic acid:ACN (20:80)	Gradient	100	1.5	11.7	DAD (306 nm)	0.002	0.006	0.01-5	0.8-7.0 (intra-day); 1.2-8.5 (inter-day)	Plasma, tissues
(26)	C18, 5 μm, 150 x 4.6 mm	H ₂ O:ACN:Acetic acid (66:33.9:0.1, pH 3.4)	Isocratic	20	0.5	7.1	DAD (306 nm)	n.a.	0.1	0.1-4.4; 4.4-65.7	3.8-8.9 (intra-day); 4.7-10.6 (inter-day)	Wine
(27)	C18, 5 μm, 250 x 4 mm	ACN:PBS 30 mM (30:70)	Isocratic	30	1	5.5	DAD (320 nm)	0.01	0.02	0.02-4.4	1.2-4.5 (intra-day); 2.0-3.9 (inter-day)	Plasma
(28)	C18, 5 μm, 250 x 4.6 mm	(A) formic acid (B) MeOH (C) H ₂ O	Gradient	20	1	1.4 (DAD); 11.1 (fluo)	DAD (323 nm); fluorescence (330/374 nm)	0.09 (DAD); 0.04 (fluo)	0.4 (DAD); 0.2 (fluo)	0.9-22 (DAD); 1-22 (fluo)	n.a.	Wine
(29)	C18, 5 μm, 250 x 4.6 mm	MeOH:phosphate buffer adjusted to pH 6.8 with 0.5% orthophosphoric acid in water (63:37)	Isocratic	50	1	3.9	DAD (306 nm)	0.03	0.04	0.04-28	0.5-1.0 (intra-day); 0.6-2.1 (inter-day)	Plasma
(30)	C18, 5 μm, 250 x 4.6 mm	(A) 2% aqueous acetic acid (B) ACN	Gradient	5	1	22.2	DAD (306 nm)	0.02	n.a.	0.04-219	0.1 (intra-day); 0.4 (inter-day)	Wine

n.a. – not available.

Table S2 – Composition of HBSS, FaSSIF and FeSSIF media.

Composition	Concentration (mg L ⁻¹)
HBSS (pH 7.4)	
Calcium Chloride	140
Magnesium Chloride	100
Magnesium Sulfate	100
Potassium Chloride	400
Potassium Phosphate monobasic	60
Sodium Bicarbonate	350
Sodium Chloride	8000
Sodium Phosphate dibasic	48
D-Glucose	1000
FaSSIF (pH 6.5)	
Sodium hydroxide	420
Sodium dihydrogen phosphate monohydrate	3950
Sodium chloride	6190
Sodium taurocholate	1680
Lecithin	560
FeSSIF (pH 5.0)	
Sodium hydroxide	4040
Glacial Acetic Acid	8650
Sodium chloride	11870
Sodium taurocholate	8400
Lecithin	2800