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Article

Amorphous System of Hesperetin and Piperine—Improvement of Apparent Solubility, Permeability, and Biological Activities

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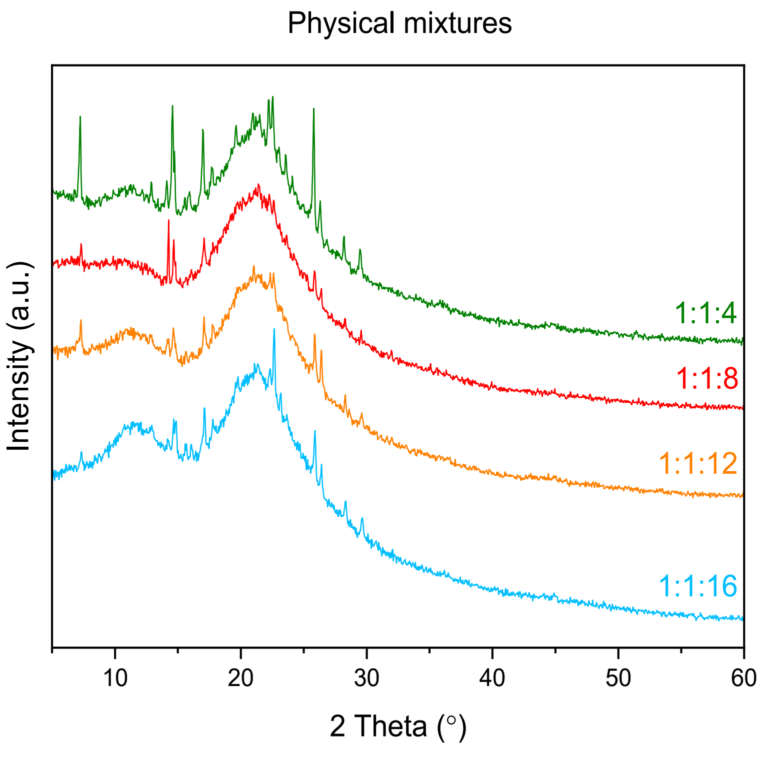


Figure S1. Diffractograms physical mixtures of the systems.

The FT-IR pattern of raw hesperetin is characterized by peaks such as 3499 cm-1 (O-H stretching), 1646 cm-1 (C=O stretching) strong bands at 1609-1294 cm-1 (C=C stretching of aromatic ring), 1465-1230 cm-1 (C-O stretching between the aromatic ring and hydroxyl/methoxy groups), 900-800 cm-1 (C-H rocking of aromatic ring) [51, 52]. Raw piperine showed characteristic peaks at 2939 cm−1 (C-H stretching), 1633 cm−1 (N-H bending), 1581 cm−1 (N-C=O stretching in carbonyl amide), 1506 cm-1 (C-NH asymmetric bending and C=C stretching of aromatic ring), 1437 cm-1 (C=CH2 CH2 deformation and bending), 1368 cm-1 (C-H bending), 1310 cm-1 (C-N asymmetric stretching), 1252 cm-1

(C-O stretching in methylenedioxy group), 927 cm-1 (C-O vibration in methylenedioxy-phenyl) [24,53,54,58]. The FT-IR spectra of pure Kollidon VA64 is described by the peak at 1730 cm−1 (vinyl acetate), 1668 cm−1 (C=O stretching) as well as 1290 cm−1 (C-N vibration) [55-57].

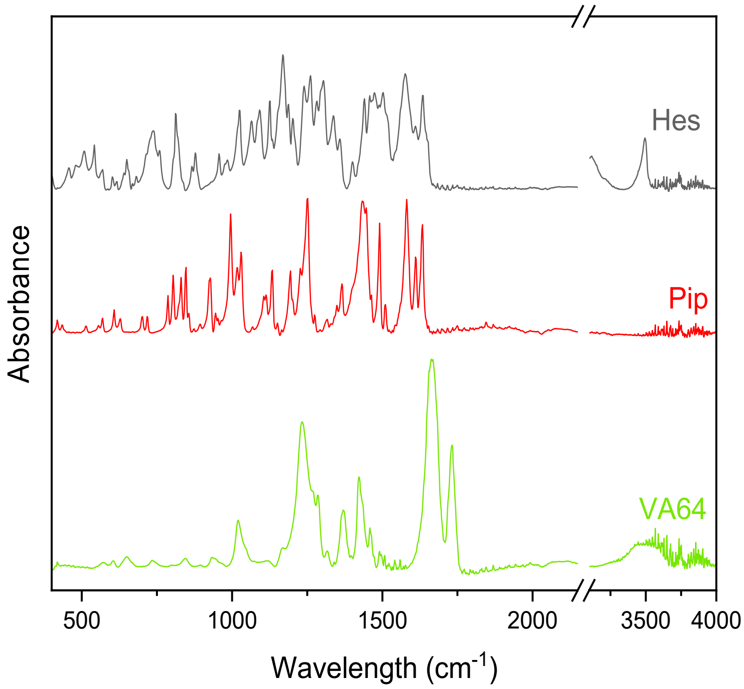
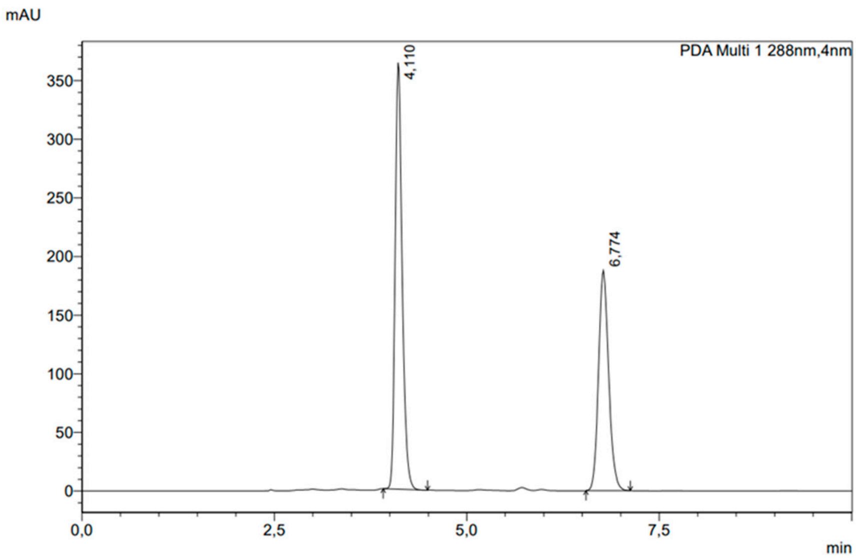
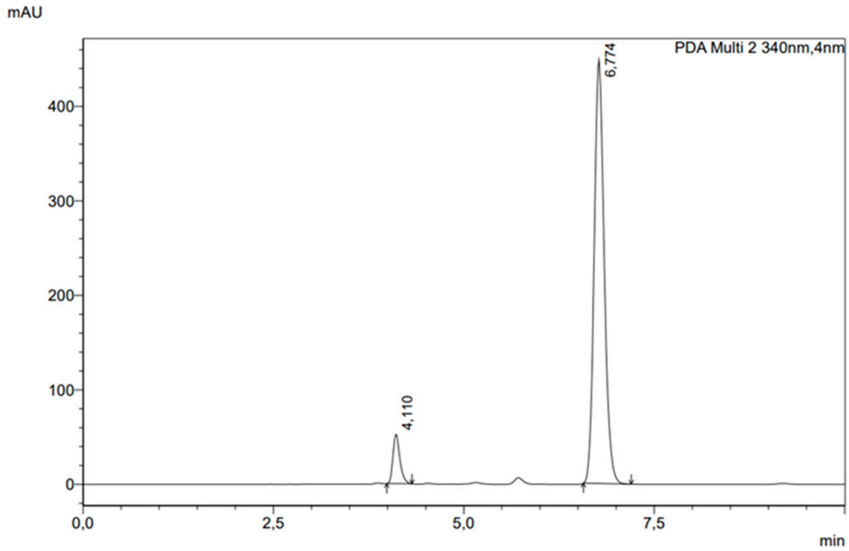


Figure S2. FT-IR/ATR spectra of raw compounds



(a)



(b)

Figure S3. Chromatograms of hesperetin (**a**) and piperine (**b**).

Chromatographic conditions

- Stationary phase: Dr. Maisch ReproSil-Pur Basic-C18 100 Å column, 5 µm particle size, 250 × 4.60 mm
- Mobile phase: methanol/0.1% acetic acid (80:20 v/v)
- Column temperature: 30 °C
- Flow rate: 1.0 mL·min^{-1}

Table S1. Validation parameters of HPLC-DAD method for concentration determination of hesperetin and piperine

Hesperetin	
Parameter	Hesperetin dissolved in 50% DMSO; Injection volume 10 µl
Linearity range (mg·mL^{-1})	0.00008 – 0.48
Correlation coefficient (r)	0.9999
a ± S_a	38446721 ± 196766
b ± S_b	insignificant (α=0.05)
LOD (mg·mL^{-1})	0.0075
LOQ (mg·mL^{-1})	0.0228
Retention Time	4.11
Piperine	
Parameter	Piperine dissolved in 50% DMSO; Injection volume 10 µl
Linearity range (mg·mL^{-1})	0.00008 – 0.16
Correlation coefficient (r)	0.9975
a ± S_a	68194881 ± 1815271

b ± S_b	insignificant (α=0.05)
LOD (mg·mL^{-1})	0.0136
LOQ (mg·mL^{-1})	0.0411
Retention Time (min)	6.77

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