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Supplementary Material



HPLC and HPLC-MS Analysis of Intestinal Elimination and Phase 2 Metabolism of 4'-hydroxy-4-Methoxychalcone and its Bis-Mannich Analog In The Rat

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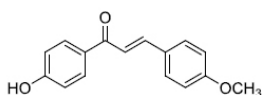
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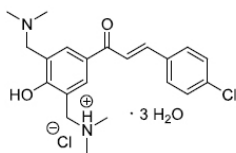
2.2.. Synthesis of compounds 1 and 2

The 4'-hydroxy-4-methoxychalcone (**1**) [37] and the bis-Mannich chalcone (**2**) [30] were synthesized as published earlier. Structural characterization of the samples supported by IR and NMR. Infrared (IR) spectra were recorded on a Bruker IFS-55 FT, ¹H nuclear magnetic resonance (NMR) spectra on a Bruker Avance III 500 (500.15 MHz for ¹H) spectrometer (Bruker Optik GmbH, Ettlingen, Germany).

Chalcone **1** was synthesized by Claisen-Schmidt condensation as described previously by Kumar et al. [37] with slight modifications [30]. Equimolar portions of 4-hydroxyacetophenone (0.01 mol) and 4-methoxybenzaldehyde (0.01 mol) were added to 15 mL of 40% NaOH methanol solution (w/v). The reaction mixture was stirred at 64 °C for 13 h when TLC indicated the end of the reactions. Then, cold water (5 mL) was added to the media and acidified with 10% hydrochloric acid (w/v) until pH 3. The resulting yellow powder obtained was crystallized from methanol to yield the hydroxychalcone.



1



2

(2*E*)-1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**1**): m.p.: 187-190 °C; IR (KBr) ν/cm^{-1} 3130, 1647, 1600, 1177; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8,05 (d, 2H, J 8,73), 7,83 (d, 2H, J 8,22), 7,78 (d, 1H, J 15,41, H _{β}), 7,65 (d, 1H, J 15,41, H _{α}), 7,01 (d, 2H, J 8,22), 6,89 (d, 2H, J 8,73), 3,82 (s, 3H, OCH₃).

The Mannich base **2** was synthesized from 4-chloro-4'-hydroxychalcone using the Mannich reagent *N,N*-dimethylmethyleiminium chloride, also known as Eschenmoser's salt, previously prepared by a literature procedure [30]. The Mannich reagent *N,N*-dimethylmethyleiminium chloride (2.0 mmol) was dissolved in a solution of hydroxychalcone **1** (1.0 mmol) in acetonitrile (10 mL), and the mixture was heated under reflux for 78 h. The solution was concentrated. Then, a hydrogen chloride solution was added in diethyl ether to form the corresponding hydrochloride salt **2**. The yellow solid was crystallized from a mixture of water and ethyl acetate.

(2*E*)-1-[3,5-bis[(dimethylamino)methyl]-4-hydroxyphenyl]-3-(4-chlorophenyl)prop-2-en-1-one hydrochloride (**2**): mp 110-113 °C; IR (KBr) ν/cm^{-1} 3418, 2703, 1657, 1602; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.12 (s, 2H), 7.99 (d, 1H, J 15.41, H _{β}), 7.92 (d, 2H, J 8,43), 7.65 (d, 1H, J 15.41, H _{α}), 7.52 (d, 2H, J 8.43), 4.14 (s, 4H), 2.64 (s, 12H).

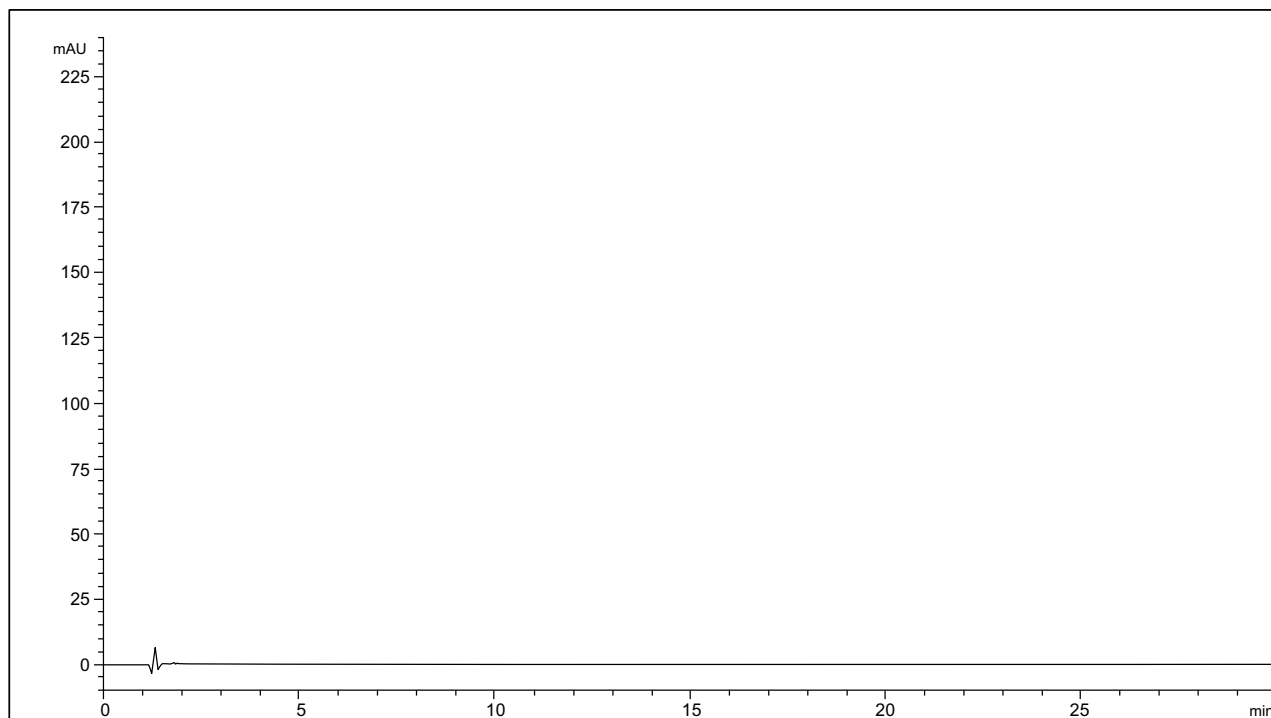


Fig. (S1A). HPLC-UV-Vis chromatogram (Method 1) of the blank perfusate (with 3.5 v/v% PEG-400) generated in rat small intestine luminal perfusion experiments without compound **1**.

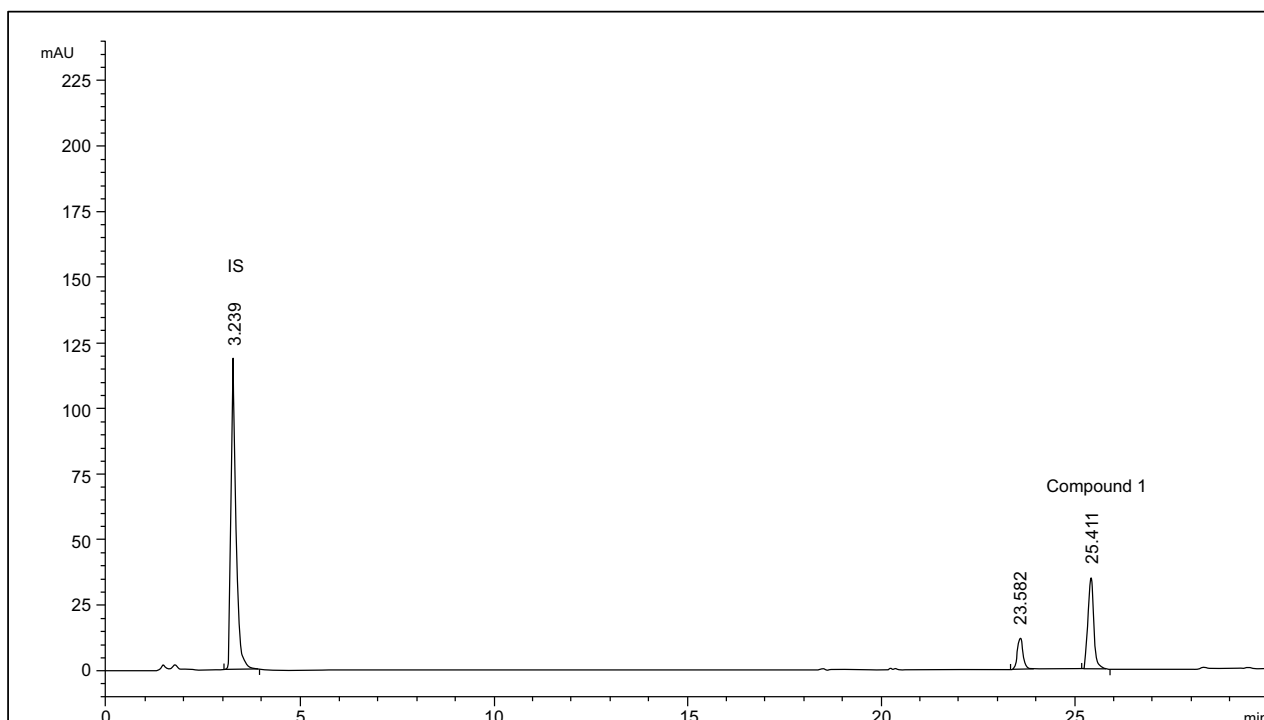


Fig. (S1B). HPLC-UV-Vis chromatogram (Method 1) of the perfusate (with 3.5 v/v % PEG-400) generated in rat small intestine luminal perfusion experiments with compound **1** at the 45th-minute timepoint of the perfusion period. (t_r 23.582: unidentified contaminant of **1**).

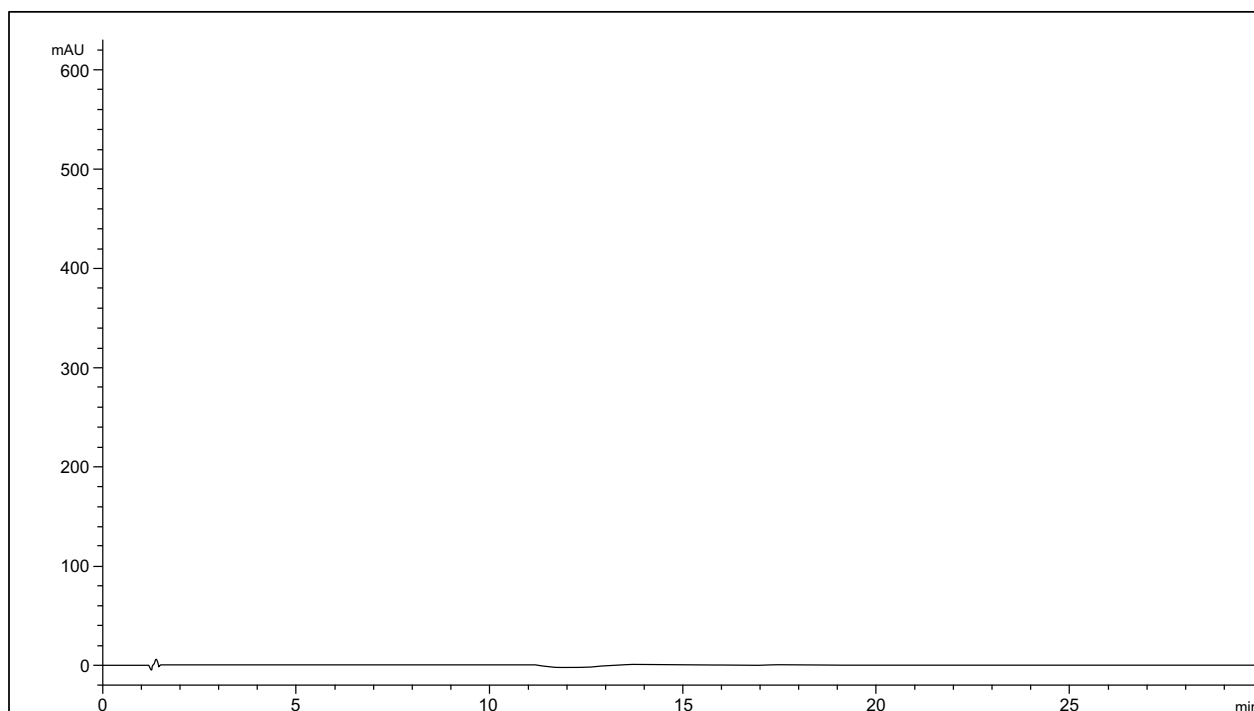


Fig. (S2A). HPLC-UV-Vis chromatogram (Method 2) of the blank perfusate generated in rat small intestine luminal perfusion experiments without compound **2**.

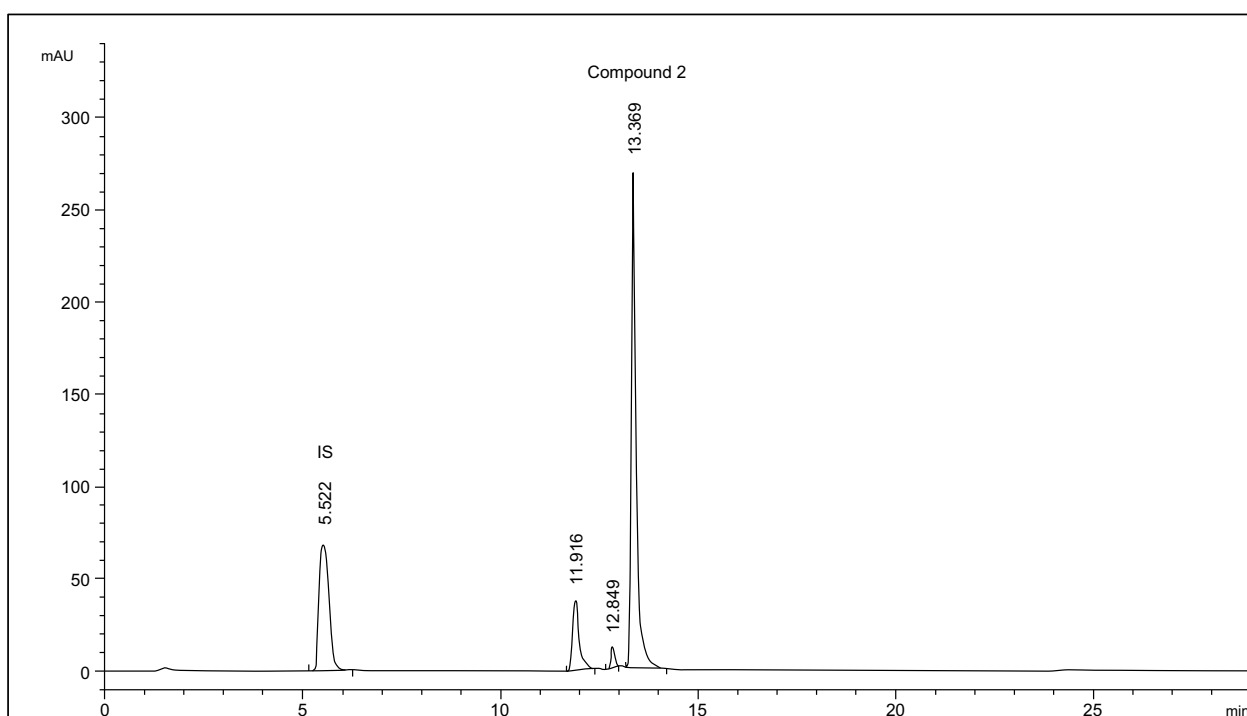


Fig. (S2B). HPLC-UV-Vis chromatogram (Method 2) of the perfusate generated in rat small intestine luminal perfusion experiments with compound **2** at the 45th-minute timepoint of the perfusion period. (t_R 12.849: unidentified contaminant of **2**; t_R 11.916: unidentified metabolite of **2**)

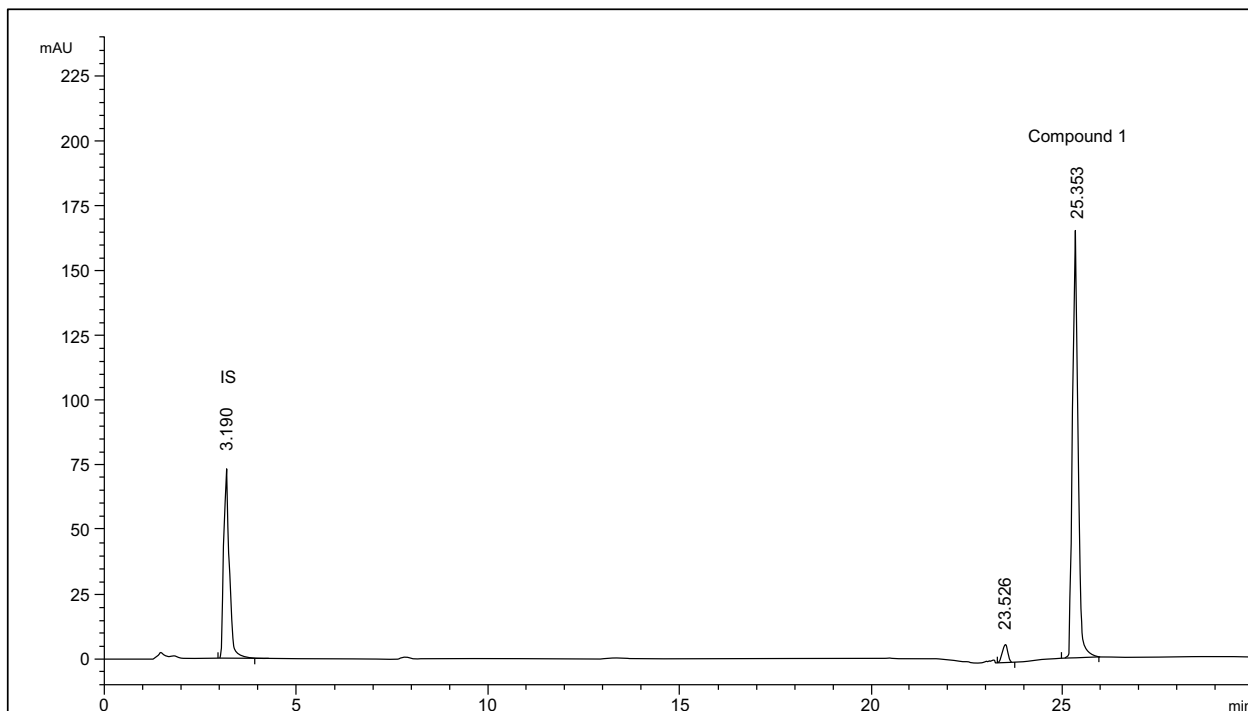


Fig. (S3A). HPLC-UV-Vis system suitability chromatogram (Method 1) of compound **1**. (t_R 23.526: unidentified contaminant of **1**).

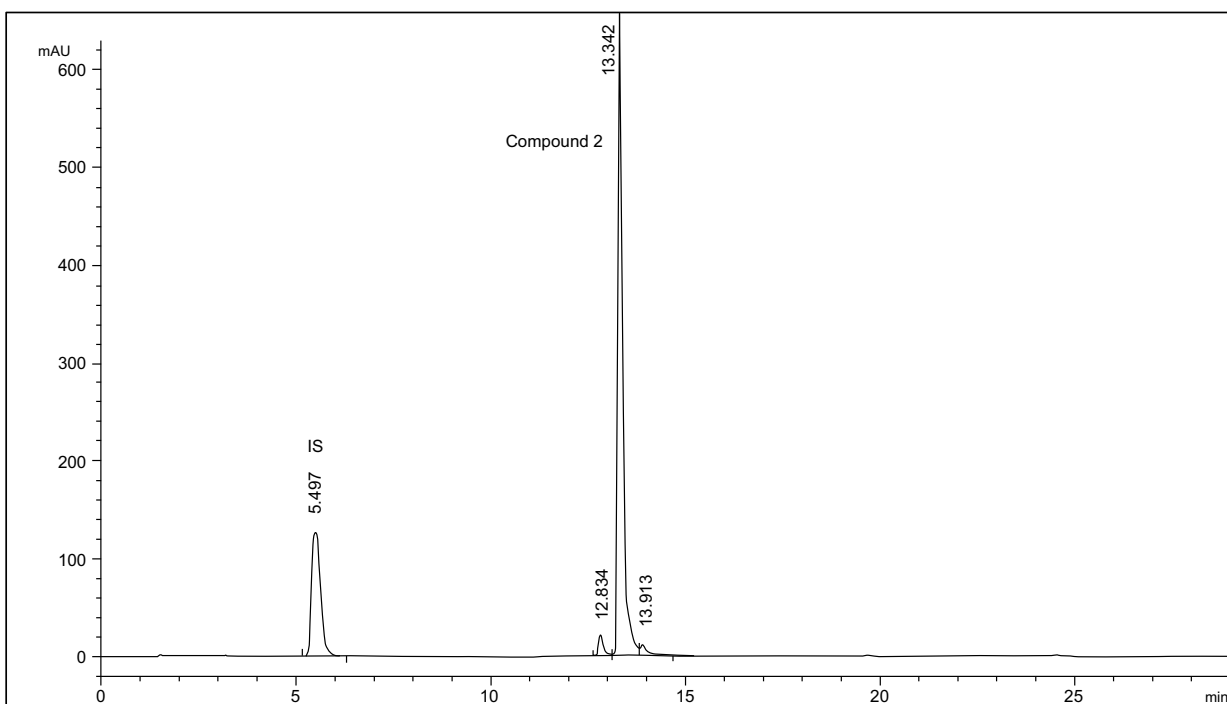


Fig. (S3B). HPLC-UV-Vis system suitability chromatogram of compound **2**. (t_R 12.834 and t_R 13.913: unidentified contaminants of **2**)

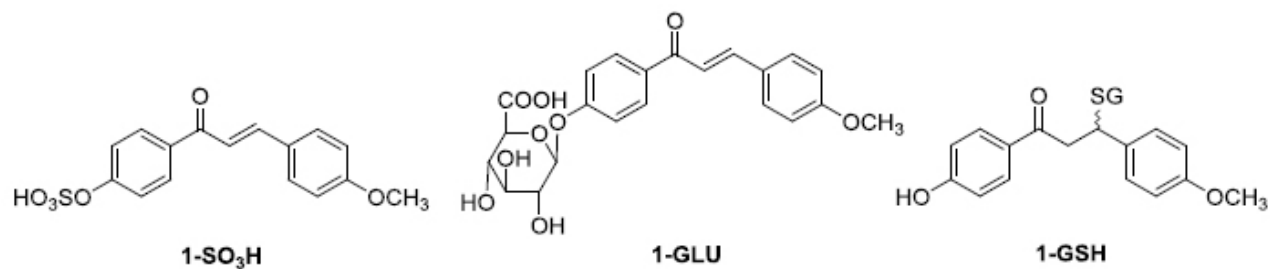


Fig. (S4). Structure of the sulfate (1-SO₃H), glucuronic acid (1-GLU), and glutathione (1-GSH) conjugates of chalcone 1.

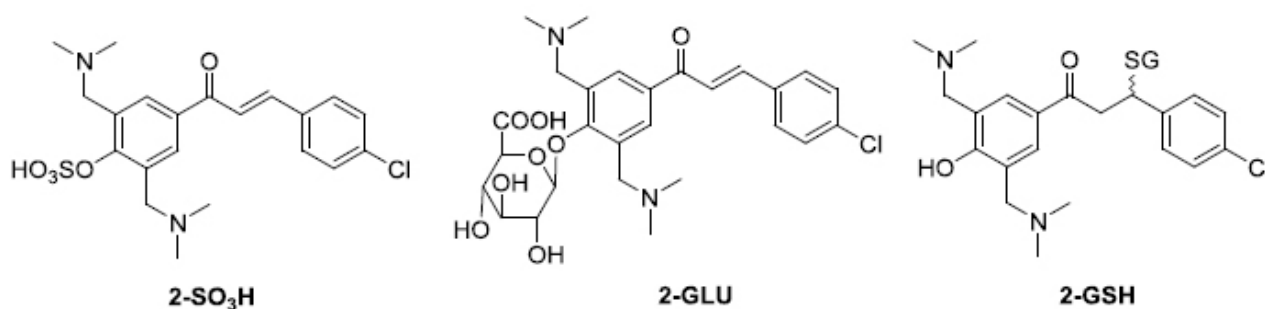


Fig. (S5). Structure of the sulfate (2-SO₃H), glucuronic acid (2-GLU), and glutathione (2-GSH) conjugates of chalcone 2.

Table S1. Intermediate precision data of 1

c (mg/mL)	Weighing	Dilution	A _{I.S.}	A ₁	A ₁ /A _{I.S.}	c (mg/mL)	c (mean) (mg/mL)
0.16 (1st day)	1	2	970.5	17448.0	17.978	0.1646	0.1575
			969.2	17253.1	17.801	0.1630	
			960.4	16859.2	17.554	0.1607	
	2	3	989.6	16950.9	17.129	0.1568	
			999.2	16709.9	16.723	0.1530	
			1003.1	16108.1	16.058	0.1469	
0.16 (2nd day)	1	2	979.0	16665.8	17.023	0.1558	0.1579
			999.0	16609.2	16.626	0.1522	
			989.0	16545.2	16.729	0.1531	
	2	3	1057.4	18726.8	17.710	0.1621	
			1055.3	18724.6	17.743	0.1624	
			1052.6	18565.9	17.638	0.1615	
0.16 (3rd day)	1	2	1098.0	17020.9	15.502	0.1418	0.1520
			1052.0	17050.8	16.208	0.1483	
			1062.0	17110.0	16.111	0.1474	
	2	3	1074.0	18476.6	17.204	0.1575	
			1071.8	18455.8	17.219	0.1576	
			1060.9	18453.7	17.394	0.1592	
Results		Mean	1024.6	17429.7	17.020	0.1558	
		SD	43.9	879.5	0.706	0.0065	
		%RSD	4.3	5.0	4.146	4.1704	
c (mg/mL)	Weighing	Dilution	A _{I.S.}	A ₁	A ₁ /A _{I.S.}	c (mg/mL)	c (mean) (mg/mL)

0.02 (1st day)	1	1	1040.8	2316.9	2.226	0.0196	0.0193	
			1036.6	2324.8	2.243	0.0197		
			1034.1	2362.1	2.284	0.0201		
	2	1120.0	2398.4	2.141	0.0188			
		1108.0	2381.6	2.149	0.0189			
		1113.5	2341.9	2.103	0.0185			
0.02 (2nd day)	1	1	1193.8	2299.9	1.927	0.0168	0.0176	
			1193.1	2297.9	1.926	0.0168		
			1190.4	2285.2	1.920	0.0168		
	2	3	1107.0	2321.6	2.097	0.0184		
			1092.0	2311.5	2.117	0.0186		
			1099.0	2308.9	2.101	0.0184		
0.02 (3rd day)	1	1	1052.1	2251.3	2.140	0.0188	0.0187	
			1055.6	2253.6	2.135	0.0187		
			1050.0	2253.0	2.146	0.0188		
	2	3	1020.0	2198.6	2.155	0.0189		
			1038.9	2200.7	2.118	0.0186		
			1052.8	2190.2	2.080	0.0182		
Results			Mean	1088.8	2294.3	2.112	0.0185	
			SD	56.9	60.4	0.101	0.0009	
			%RSD	5.2	2.6	4.789	5.0241	
c (mg/mL)	Weighing	Dilution	A_{1s}	A₁	A₁/A_{1s}	c (mg/mL)	c (mean) (mg/mL)	
0.004 (1st day)	1	1	1096.0	465.6	0.425	0.0030	0.0032	
			1089.0	473.7	0.435	0.0031		
			1085.0	481.3	0.444	0.0032		
		2	1033.5	451.2	0.437	0.0031		
			1029.5	454.3	0.441	0.0032		
			1025.0	501.1	0.489	0.0036		
0.004 (2nd day)	1	1	1149.9	528.0	0.459	0.0033	0.0033	
			1147.3	527.7	0.460	0.0033		
			1148.5	514.3	0.448	0.0032		
		3	1158.0	522.0	0.451	0.0032		
			1148.0	529.0	0.461	0.0033		
			1152.0	519.0	0.451	0.0032		
0.004 (3rd day)	2	2	1139.2	510.0	0.448	0.0032	0.0032	
			1138.4	508.9	0.447	0.0032		
			1134.6	507.7	0.447	0.0032		
		3	1105.0	457.1	0.414	0.0029		
			1004.2	455.5	0.454	0.0033		
			1003.2	452.6	0.451	0.0032		
Results			Mean	1099.2	492.2	0.448	0.0032	
			SD	56.2	30.0	0.016	0.0001	
			%RSD	5.1	6.1	3.527	4.5276	

Table S2. Intermediate precision data of 2.

c (mg/mL)	Weighing	Dilution	A_{1s}	A₂	A₂/A_{1s}	c (mg/mL)	c (mean) (mg/mL)
0.25 (1st day)		2	1525.7	9899.7	6.489	0.2647	0.2632
			1530.7	9810.7	6.409	0.2614	
			1528.6	9970.2	6.522	0.2661	
	2	3	1639.3	10572.8	6.450	0.2631	
			1640.3	10552.9	6.434	0.2624	
			1642.4	10536.5	6.415	0.2617	

0.25 (2nd day)	1	1	1493.3	9879.6	6.616	0.2699	0.2686	
			1496.4	9932.4	6.638	0.2708		
			1519.1	9946.6	6.548	0.2671		
	2	3	1498.7	10181.5	6.794	0.2772		
			1522.5	10072.0	6.615	0.2699		
			1560.0	9831.8	6.302	0.2571		
0.25 (3rd day)		2	1682.8	10103.9	6.004	0.2449	0.2530	
			1684.7	10417.5	6.184	0.2522		
			1689.7	10463.3	6.192	0.2526		
	2	3	1602.1	9886.5	6.171	0.2517		
			1614.4	10223.7	6.333	0.2583		
			1619.0	10261.5	6.338	0.2585		
Results			Mean	1582.76	10141.28	6.414	0.2616	
			SD	70.38	268.82	0.198	0.0081	
			%RSD	4.45	2.65	3.086	3.0928	
c (mg/mL)	Weighing	Dilution	A _{1s}	A ₂	A ₂ /A _{1s}	c (mg/mL)	c (mean) (mg/mL)	
0.05 (1st day)	1	1	1668.3	2286.1	1.370	0.0555	0.0561	
			1690.5	2347.1	1.388	0.0562		
			1691.9	2338.7	1.382	0.0560		
		2	1468.6	2057.4	1.401	0.0567		
			1469.5	2031.7	1.383	0.0560		
			1459.1	2020.8	1.385	0.0561		
0.05 (2nd day)	1	1	1578.7	2055.1	1.302	0.0527	0.0519	
			1577	2048.4	1.299	0.0526		
			1577.1	2045.9	1.297	0.0525		
		3	1713.2	2185.3	1.276	0.0516		
			1723.6	2186	1.268	0.0513		
			1715.6	2161	1.260	0.0509		
0.05 (3rd day)	2	2	1670.1	2101.8	1.258	0.0509	0.0533	
			1688.4	2169.7	1.285	0.0520		
			1690.7	2168.3	1.282	0.0519		
		3	1675.9	2260.3	1.349	0.0546		
			1711.6	2331.6	1.362	0.0551		
			1711.1	2327.9	1.360	0.0551		
Results			Mean	1637.8	2173.5	1.328	0.0538	
			SD	92.1	117.6	0.051	0.0021	
			%RSD	5.6	5.4	3.865	3.9038	
c (mg/mL)	Weighing	Dilution	A _{1s}	A ₂	A ₂ /A _{1s}	c (mg/mL)	c (mean) (mg/mL)	
0.005 (1st day)	1	1	1523.6	241.3	0.158	0.0059	0.0055	
			1525.2	233.4	0.153	0.0057		
			1526.6	234.8	0.154	0.0057		
		2	1593.2	221.9	0.139	0.0052		
			1594.7	225.8	0.142	0.0052		
			1594.1	223.3	0.140	0.0052		
0.005 (2nd day)	1	1	1596.3	219.9	0.138	0.0051	0.0050	
			1597.5	222.4	0.139	0.0052		
			1601.1	219.5	0.137	0.0051		
		2	1708.9	239.1	0.140	0.0052		
			1704.6	222.2	0.130	0.0048		
			1674.6	226.6	0.135	0.0050		

0.005 (3rd day)	2	1	1682.4	235.9	0.140	0.0052	0.0051
			1716.4	239.4	0.139	0.0052	
			1716.7	234.2	0.136	0.0050	
		3	1643.0	223.7	0.136	0.0050	
			1646.3	222.2	0.135	0.0050	
			1693.2	227.9	0.135	0.0050	
Results		Mean	1629.9	228.5	0.140	0.0052	
		SD	66.8	7.4	0.007	0.0003	
		%RSD	4.1	3.2	5.208	5.7486	

Table S3. Data for accuracy of 1

c (mg/mL)	Dilution	A _{I.S.}	A ₁	A ₁ /A _{I.S.}	c (mg/mL)
0.08	1	1029.4	8855.5	8.603	0.0783
	2	1035.6	8914.2	8.608	0.0783
	3	968.9	8271.9	8.537	0.0777
	4	931.3	7941.3	8.527	0.0776
	5	939.5	8245.3	8.776	0.0799
	Mean	980.9	8445.6	8.610	0.0784 (98.00%*)
	SD	49.1	422.0	0.100	0.0009
	%RSD	5.0	5.0	1.159	1.1726
c (mg/mL)	Dilution	A _{I.S.}	A ₁	A ₁ /A _{I.S.}	c (mg/mL)
0.032	1	978.6	3306.4	3.379	0.0302
	2	876.2	2987.5	3.410	0.0305
	3	1012.9	3568.1	3.523	0.0315
	4	1089.4	3789.2	3.478	0.0311
	5	1061.8	3678.2	3.464	0.0310
	Mean	1003.8	3465.9	3.451	0.0309 (96.56%*)
	SD	83.2	321.8	0.057	0.0005
	%RSD	8.3	9.3	1.651	1.7002
c (mg/mL)	Dilution	A _{I.S.}	A ₁	A ₁ /A _{I.S.}	c (mg/mL)
0.012	1	1051.8	1475.1	1.402	0.0120
	2	1050.7	1430.1	1.361	0.0116
	3	955.7	1310.3	1.371	0.0117
	4	896.0	1229.7	1.372	0.0117
	5	955.7	1310.3	1.371	0.0117
	Mean	982.0	1351.1	1.376	0.0118 (98.33%*)
	SD	67.8	99.6	0.016	0.0001
	%RSD	6.9	7.4	1.140	1.2281

c, calculated concentration (mg/mL) I.S., internal standard: 2,5-dihydroxybenzoic acid *Calculated from six injections **Measured concentration expressing in the percentage of the expected concentration

Table S4. System suitability data of Method 1 and Method 2.

c (mg/mL)	Dilution	A _{I.S.}	A ₂	A ₂ /A _{I.S.}	c (mg/mL)
0.15	1	1702.6	6349.3	3.729	0.1519
	2	1695.6	6375.6	3.760	0.1532
	3	1696.1	6376.7	3.760	0.1531
	4	1697.5	6352.8	3.742	0.1524
	5	1698.0	6348.3	3.739	0.1523
	Mean	1698.0	6360.5	3.746	0.1526 (101.73%*)
	SD	2.8	14.4	0.014	0.0006
	%RSD	0.2	0.2	0.361	0.3627
c (mg/mL)	Dilution	A _{I.S.}	A ₂	A ₂ /A _{I.S.}	c (mg/mL)

0.075	1	1688.9	3177.6	1.881	0.0764
	2	1785.6	3329.5	1.865	0.0757
	3	1772.5	3229.9	1.822	0.0739
	4	1693.0	3296.0	1.947	0.0790
	5	1691.0	3202.6	1.894	0.0769
	Mean	1726.2	3247.1	1.882	0.0764 (101.87%*)
	SD	48.5	63.8	0.045	0.0019
	%RSD	2.8	2.0	2.409	2.4260
c (mg/mL)	Dilution	A_{I.S.}	A₂	A₂/A_{I.S.}	c (mg/mL)
0.025	1	1486.6	997.7	0.671	0.0269
	2	1591.2	999.6	0.628	0.0251
	3	1511.5	997.5	0.660	0.0264
	4	1597.7	1001	0.627	0.0251
	5	1494.8	997.1	0.667	0.0267
	Mean	1536.4	998.6	0.651	0.0261 (104.40%*)
	SD	53.8	1.7	0.022	0.0009
	%RSD	3.5	0.2	3.315	3.3835

c, calculated concentration (mg/mL) I.S., internal standard: 2,5-dihydroxybenzoic acid *Calculated from six injections **Measured concentration expressing in the percentage of the expected concentration

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