

ESI: UHPLC method

Retention time (n = 6)

Chromophore	Retention time (min)	std dev (min)
Fisetin	3.714	0.012
Sulfuretin	4.420	0.012
Luteolin	4.787	0.013
Genistein	4.919	0.011
Apigenin	5.462	0.012
Chrysoeriol	5.622	0.011
Diosmetin	5.685	0.010
Alizarin	6.287	0.010
Prunetin	6.738	0.009

Equation, calculated slope error and R² values of the calibration curves (Peak Height (AU) vs Concentration (µg ml⁻¹), (7 concentrations used, n = 5)

Chromophore	Equation	slope std error	R ² value
Fisetin	y = 0.0143x	3.51E-04	0.9958
Sulfuretin	y = 0.0124x	8.84E-05	0.9997
Luteolin	y = 0.0226x	1.95E-04	0.9995
Genistein	y = 0.0460x	1.71E-04	0.9999
Apigenin	y = 0.0121x	7.60E-04	0.9729
Chrysoeriol	y = 0.0148x	1.06E-03	0.9653
Diosmetin	y = 0.0203x	4.93E-05	0.9999
Alizarin	y = 0.0328x	9.98E-04	0.9935
Prunetin	y = 0.0278x	1.33E-03	0.9842

Average signal (peak height) of retention time \pm 0.25 min in blank runs (n = 3) used as noise in LoD and LoQ calculations.

Chromophore	Peak height	std dev
Fisetin	1.25E-03	2.24E-05
Sulfuretin	1.50E-03	4.94E-05
Luteolin	1.46E-03	4.55E-05
Genistein	1.48E-03	4.34E-05
Apigenin	1.61E-03	3.44E-05
Chrysoeriol	1.67E-03	3.64E-05
Diosmetin	1.69E-03	3.70E-05
Alizarin	1.92E-03	9.16E-05
Prunetin	2.29E-03	8.99E-05

Limit of detection (LoD) and limit of quantification (LoQ) calculated using the signal-to-noise method

Chromophore	LoD ($\mu\text{g ml}^{-1}$)	std dev	LoQ ($\mu\text{g ml}^{-1}$)	std dev
Fisetin	0.29	0.03	0.88	0.03
Sulfuretin	0.40	0.03	1.21	0.03
Luteolin	0.21	0.03	0.65	0.03
Genistein	0.11	0.03	0.32	0.03
Apigenin	0.44	0.07	1.33	0.07
Chrysoeriol	0.37	0.07	1.13	0.07
Diosmetin	0.27	0.02	0.83	0.02
Alizarin	0.19	0.06	0.59	0.06
Prunetin	0.27	0.06	0.83	0.06

ESI: Figure 3

Chromophore	20 µg ml ⁻¹		10 µg ml ⁻¹		5 µg ml ⁻¹		2.5 µg ml ⁻¹		1 µg ml ⁻¹		0.5 µg ml ⁻¹		0.1 µg ml ⁻¹	
	% Recovery	std dev	% Recovery	std dev	% Recovery	std dev	% Recovery	std dev	% Recovery	std dev	% Recovery	std dev	% Recovery	std dev
Fisetin	93.10	4.17	96.08	6.07	96.50	9.47	97.22	8.79	87.83	6.96	99.71	13.04	87.21	17.65
Sulfuretin	94.90	4.37	96.57	6.95	97.29	6.87	100.11	4.57	86.25	7.15	87.74	4.77	103.29	17.33
Luteolin	95.83	5.18	97.76	6.39	97.77	6.42	97.57	6.49	87.39	6.37	90.11	5.93	102.86	13.19
Genistein	94.64	6.40	95.24	6.17	98.93	6.70	101.27	6.71	86.56	6.95	91.64	7.77	106.16	17.38
Apigenin	91.41	8.26	97.63	6.73	98.54	6.80	100.38	7.24	89.40	6.87	89.47	8.16	100.26	14.38
Chrysoeriol	97.67	8.63	96.00	8.85	99.35	7.80	100.09	8.00	87.11	6.58	86.62	6.44	103.50	15.06
Diosmetin	98.14	7.95	98.36	6.86	97.26	6.32	96.93	7.11	85.56	7.26	88.44	3.75	106.66	18.06
Alizarin	92.17	7.90	96.33	5.06	90.08	9.35	89.52	9.87	85.06	8.66	90.67	12.42	79.77	28.38
Prunetin	97.98	12.54	88.77	12.68	108.20	17.08	111.70	17.13	94.48	8.35	92.95	9.97	108.86	19.63

Figure 3, left: Average percentage recoveries of the filtration step for all tested concentrations (n = 5).

Chromophore	Equation	slope std error (n = 5)
Fisetin	y = 0.9405x	0.0140
Sulfuretin	y = 0.9306x	0.0193
Luteolin	y = 0.9449x	0.0168
Genistein	y = 0.9452x	0.0165
Apigenin	y = 0.9469x	0.0175
Chrysoeriol	y = 0.9473x	0.0224
Diosmetin	y = 0.9236x	0.0203
Alizarin	y = 0.9144x	0.0171
Prunetin	y = 0.9998x	0.0313

Figure 3, middle: Equation and calculated slope error (n = 5) for the Peak Area_{unfiltered} vs Peak Area_{filtered} graphs.

Figure 3, right: Average Peak Area of the reconstitution solvents for all chromophores using reconstitution volume of 25 μ l (n = 3)

Chromophore	DMSO		10 % DMSO		MeOH:H ₂ O 1:1 v/v		0.1 % FA		Control	
	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev
Fisetin	584956	21910	740851	19413	646132	2321	813981	21781	322012	3870
Sulfuretin	474759	18653	601883	21878	535500	2609	667076	17748	258619	3111
Luteolin	910957	33087	1079223	42854	982944	8686	1237990	34310	478773	6975
Genistein	1760157	49858	2059121	81172	1945549	13517	2416550	63178	924830	12426
Apigenin	649227	25129	591372	7930	497595	12373	618152	15205	302260	9060
Chrysoeriol	789194	30867	595086	914	537781	7445	644735	13489	274915	5522
Diosmetin	821619	31000	1033167	55532	850510	14048	1010157	26199	444557	8377
Alizarin	1391289	51705	1827053	79932	1526886	9881	1920672	51500	747887	6731
Prunetin	1448874	18871	1114907	51880	1061709	6358	1207820	25844	611611	19195

Figure 3, right: Average Peak Area of the reconstitution solvents for all chromophores using reconstitution volume of 50 μ l (n = 3)

Chromophore	DMSO		10 % DMSO		MeOH:H ₂ O 1:1 v/v		0.1 % FA		Control	
	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev	Peak Area	std dev
Fisetin	268308	20272	301611	1647	314650	6521	383581	8149	322012	3870
Sulfuretin	234928	3873	254295	832	263701	5004	320314	6207	258619	3111
Luteolin	458603	13610	476975	1417	491980	6924	594654	12891	478773	6975
Genistein	860323	15962	952866	2356	986156	15996	1173186	24731	924830	12426
Apigenin	318031	4897	321043	2818	275524	10245	299474	6020	302260	9060
Chrysoeriol	511751	16059	358602	3640	276197	3468	316876	6894	274915	5522
Diosmetin	288485	4546	439253	1063	441505	5945	504929	11750	444557	8377
Alizarin	706257	13721	737333	1716	766978	13122	927362	18622	747887	6731

Prunetin	735502	23671	645643	11944	544794	10067	604329	14086	611611	19195
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