

Supporting Information

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Development of RP-HPLC-DAD method for quantitative analysis of quercetin and piperine in botanical extracts

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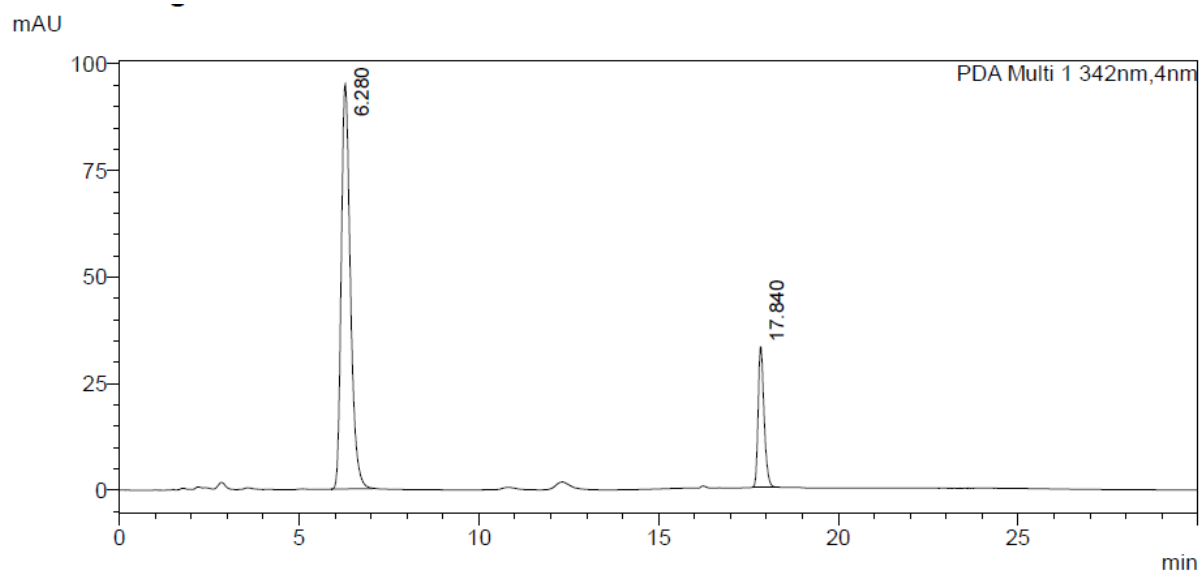


Figure S1: Representative chromatogram of quercetin and piperine standard solution at 342 nm

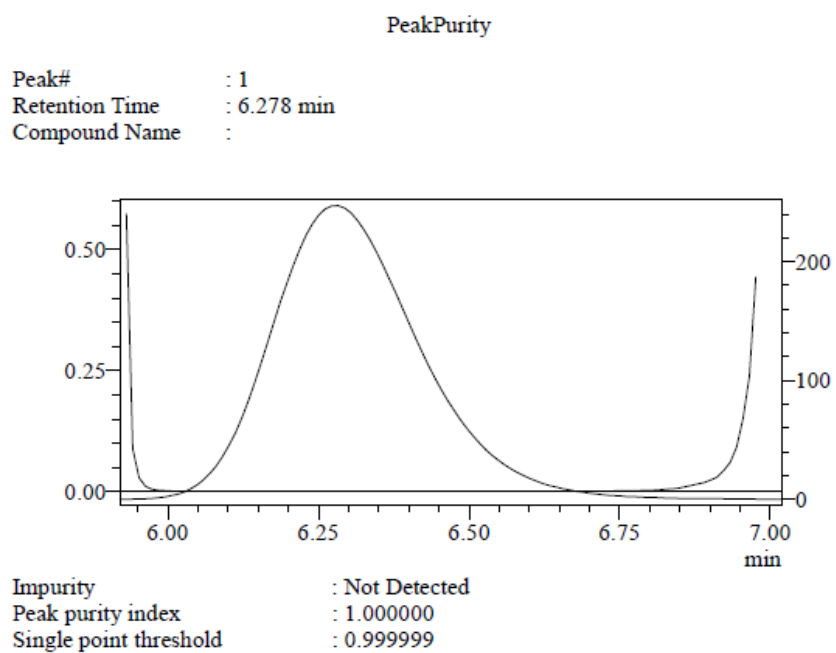
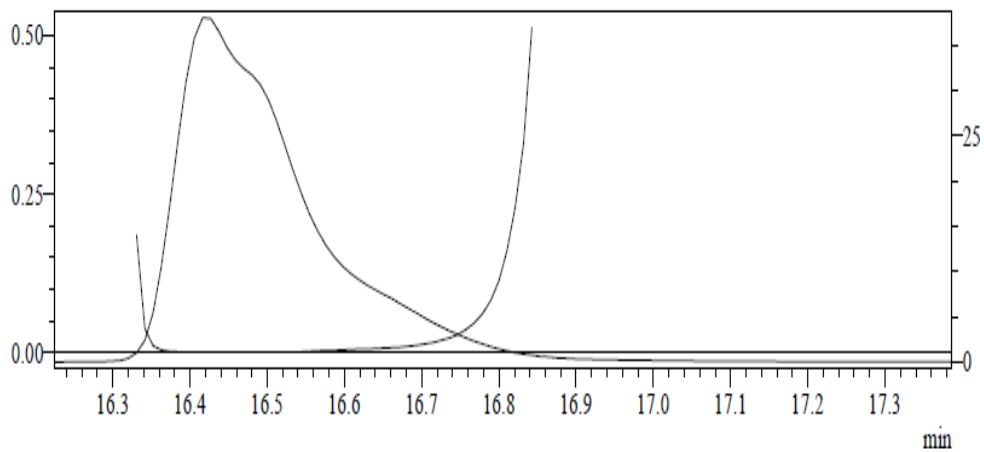


Figure S2: Peak purity of standard quercetin

PeakPurity

Peak# : 1
Retention Time : 16.422 min
Compound Name :



Impurity : Not Detected
Peak purity index : 0.999827
Single point threshold : 0.999125

Figure S3: Peak purity of standard piperine

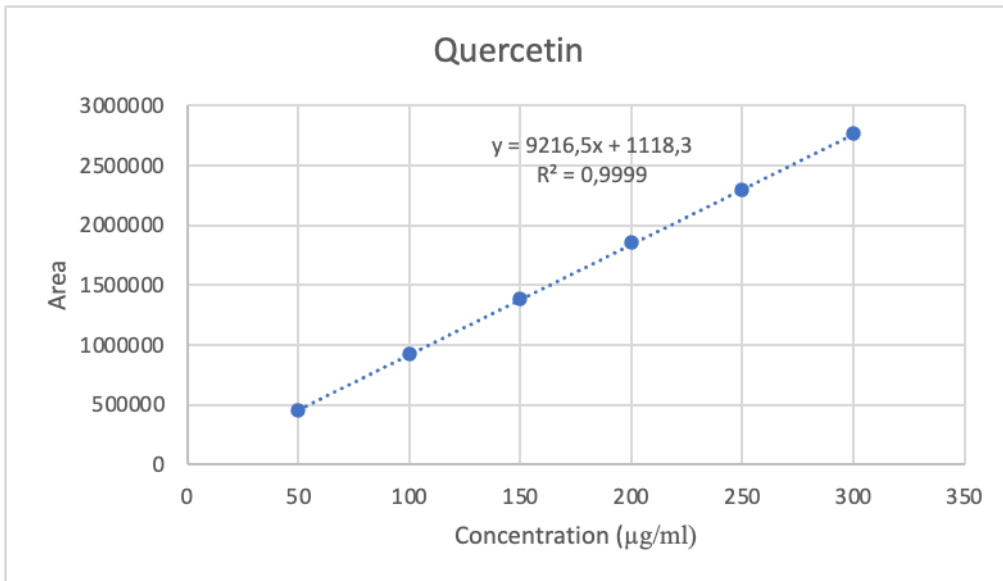


Figure S4: Calibration curve of quercetin between 50 to 300 µg m/L

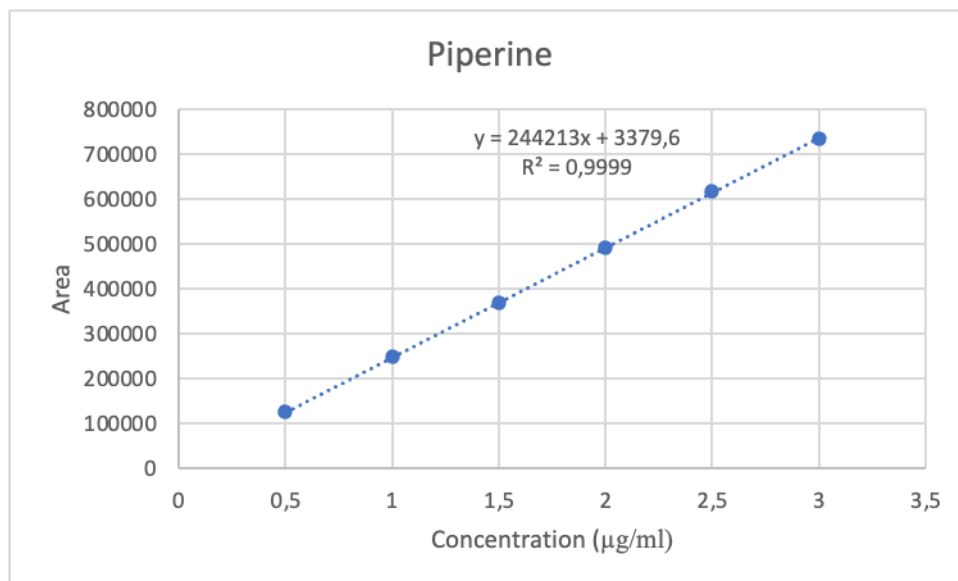


Figure S5: Calibration curve of piperine between 0.5 to 3 µg m/L

Table S1: Observation and remarks for selection of mobile phase for quercetin and piperine

Sr. No.	Trial			Observation
1	ACN : Water (60:40 v/v)			Quercetin peak was not observed
2	ACN : Water (60:40) pH: 2.6 (Adjusted by FA)			Quercetin peak was not observed
3	ACN : Water (0.3% FA) (90:10)			Quercetin peak was not observed
4	ACN : Water (30:70) pH: 2.6 by FA			Quercetin peak was not observed
5	ACN : Water (30:70) pH 4 by FA			Quercetin peak was not observed
6	MEOH : Water (0.1% FA) (65:35)			Quercetin peak was identified Retention time: 2.528 min which was near to void volume and methanol peak.
7	MEOH : Water (0.1% FA) (60:40)			Quercetin peak was identified but theoretical plate was less than 2000 Retention time: 3.894 min
8	MEOH : Water (0.1% FA) (50:50)			Quercetin peak was identified Retention time: 6.278 min Piperine peak was identified Retention time: 40.599 min
9	Time	0.1 % FA in Water	0.1 % FA in Methanol	This mobile phase ratio was still optimized because of baseline drift and higher run time.
	0.01	50.00	50.00	
	9.00	50.00	50.00	
	11.00	30.00	70.00	
	20.00	10.00	90.00	
	25.00	10.00	90.00	
	30.00	30.00	70.00	
	35.00	30.00	70.00	
	40.00	60.00	40.00	
	45.00	Stop		
10	Time	0.1 % FA in Water	0.1 % FA in Methanol	Optimized gradient program
	0.01	50.00	50.00	
	9.00	50.00	50.00	
	11.00	30.00	70.00	
	20.00	30.00	70.00	
	25.00	50.00	50.00	
	30.00	Stop		

(ACN : Acetonitrile, MEOH : Methanol, v/v, FA : Formic acid)

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Table S2: Linearity and Range data for quercetin and piperine (n=6)

Sr. No.	Quercetin			Piperine		
	Concentration (µg/mL)	Mean Peak area ± * SD	%RSD	Concentration (µg/mL)	Mean Peak area ± * SD	%RSD
1	50	453282.3 ± 17157.7	1.80	0.5	125264.8 ± 1287.173	1.02
2	100	927288.5 ± 12269.65	1.32	1.0	248962.5 ± 1767.73	0.70
3	150	1388672 ± 16051.72	1.15	1.5	367977.3 ± 3709.482	1.00
4	200	1852696 ± 26347.1	1.42	2	490826.3 ± 1863.226	0.37
5	250	2298694 ± 38451.28	1.67	2.5	616657 ± 3351.686	0.50
6	300	2763413 ± 43564.52	1.57	3.0	734822.8 ± 9123.092	1.24
		Linearity Equation			Linearity Equation	
		Y = 9216.5X + 1118.3 R ² = 0.9999			Y = 244213X + 3379.6 R ² = 0.9999	

(*Mean area of for 6 observations)

Table S3: Precision data for quercetin and piperine

Concentration ($\mu\text{g/mL}$)	Intra-day Precision		Inter-day Precision		Repeatability	
	Peak area \pm SD	%RSD	Peak area \pm SD	%RSD		
Quercetin						
50	473407 \pm 4820.31	1.01	467259 \pm 9191.98	1.96	Concentration ($\mu\text{g/mL}$)	150
150	1400312 \pm 13938.25	0.99	1397585 \pm 17048.87	1.21	Peak area \pm SD	1392004 \pm 13232.1
300	2806223 \pm 24159.06	0.86	2791919 \pm 42977.79	1.53	%RSD	0.95
Piperine						
0.5	126220 \pm 937.65	0.74	125831 \pm 1370.79	1.08	Concentration ($\mu\text{g/mL}$)	1.5
1.5	372289 \pm 3557.27	0.95	370551 \pm 5109.75	1.37	Peak area \pm SD	369644 \pm 4771.95
3	737117 \pm 4660.88	0.63	733495 \pm 9452.40	1.28	%RSD	1.29

Table S4: Accuracy data for quercetin and piperine (n=3)

Level of Recovery	Sample Concentration		Standard Concentration		Total Concentration		Amount Recovered		% Recovery \pm %RSD	
	Quercetin	Piperine	Quercetin	Piperine	Quercetin	Piperine	Quercetin	Piperine	Quercetin	Piperine
80 %	55	1	44	0.8	99	0.8	99.86	0.79	100.43 \pm 0.39	100.18 \pm 0.64
							99.36	0.80		
							99.07	0.81		
100 %	55	1	55	1	110	1	109.72	1.01	100.08 \pm 0.29	100.16 \pm 0.28
							110.29	1.00		
							110.28	1.00		
120 %	55	1	66	1.2	121	1.2	121.79	1.22	100.23 \pm 0.44	101.05 \pm 0.26
							120.71	1.23		
							121.36	1.22		

Table S5: Robustness data for quercetin and piperine

Parameter	Optimized Condition	Set Condition	Peak area (Mean \pm SD)	Peak area (Mean \pm SD)
			Quercetin	Piperine
Formic acid in water	0.1 %	0.08 %	1415368 \pm 4264.55	371853 \pm 1268.12
		0.12 %	1414541 \pm 3521.48	379854 \pm 1025.38
Formic acid in methanol	0.1 %	0.08 %	1405214 \pm 1354.57	374256 \pm 1854.45
		0.12 %	1414685 \pm 3652.16	371586 \pm 1822.54
Flow rate	1 mLmin ⁻¹	0.9 mLmin ⁻¹	1412121 \pm 2538.43	374462 \pm 1953.44
		1.1 mLmin ⁻¹	1415371 \pm 5363.07	377576 \pm 1713.77
Column oven temperature	30 °C	25 °C	1414911 \pm 2248.09	370534 \pm 3767.33
		35 °C	1414708 \pm 2274.77	376509 \pm 1930.58
Detection wavelength	342 nm	340 nm	1414438 \pm 3318.92	376598 \pm 2034.96
		344 nm	1415889 \pm 7823.69	375781 \pm 2489.20

Table S6: Assay data for quercetin and piperine

Drugs	Extract	Mean Peak Area	Amount	%RSD	%Content
Quercetin	<i>Moringa oleifera</i> (20 mg)	512391.3	0.55 mg ± 0.96	0.46	2.75 %w/w
Piperine	<i>Piper nigrum</i> (10 mg)	263124.7	0.01 mg ± 0.85	0.26	0.1 %w/w

(*Mean area of n=3)