<Supplementary materials>

Development and Validation of Novel HPLC Methods for Quantitative Determination of Vitamin D3 in Tablet Dosage Form

Muhammad Saqib Gohar [1], Taj Ur Rahman [1], Ali Bahadur [2], Ashraf Ali [3,*], Sarah Alharthi [4,5] and Nora Hamad Al-Shaalan [6]

Table S1: System suitability of the developed method.

Replicates	Peak Areas
1	67433
2	67286
3	67455
4	67238
5	67218
6	67173
Mean	67301
Standard deviation	117
RSD	0.1741

Table S2: Precision repeatability of the developed method.

Replicate	Peak Area/Absorbance
Replicate 1	67433
Replicate 2	67286
Replicate 3	67455
Replicate 4	67238
Replicate 5	67218
Replicate 6	67173
Mean	67301
Standard Dev	117
Relative Standard dev (RSD)	0.1741

Table S3: Precision Ruggedness by different analyst variation of the developed method.

Analyst	Sample	Assay %	%RSD
1	1	98.82	0.144
	2	98.56	
	3	98.60	
2	1	98.57	0.028
	2	98.54	
	3	98.60	

Table S4: Robustness of the developed method.

Flow rate	Area	SD	%RSD
2.4	68031	551	0.81
2.5	67206		
2.6	68250		
Mobile Phase composition	**Area**	**SD**	**%RSD**
82:18	66577	481	0.72
85:15	67173		
87:13	67529		

Table S5: Limit of Detection LOD of the developed method.

Sample	Conc. %	Conc. mg/ml	Abs.	Standard Deviation	Slope	Conc. (ug/ml) at LOD	Absorbance at LOD
1	80	0.00607	53707	117	7174221	0.0539	482
2	90	0.00675	60367				
3	100	0.0075	67078				
4	110	0.0082	73582				
5	120	0.0098	80315				

Table S6: Limit of Quantification LOQ of the developed method.

Sample	Conc (%)	Conc. mg/ml	Abs.	Standard Deviation	Slope	Conc. (ug/ml) at LOQ	Absorbance at LOQ
1	80	0.00607	53707	117	7174221	0.1633	1460
2	90	0.00675	60367				
3	100	0.0075	67078				
4	110	0.0082	73582				
5	120	0.0098	80315				

Table S7: Specificity Placebo Interference in the developed method.

Sample	**Peak Area**
Aerosil USP	No peak is detected
Ossein mineral complex	No peak is detected
Primojel BP	No peak is detected
Mg Stearate BP	No peak is detected
Starch	No peak is detected

Table S8: Specificity blank Interference in the developed method

Diluent	Peak Area
Diluent and mobile phase	No peak is detected at the range of λ max 292 nm.

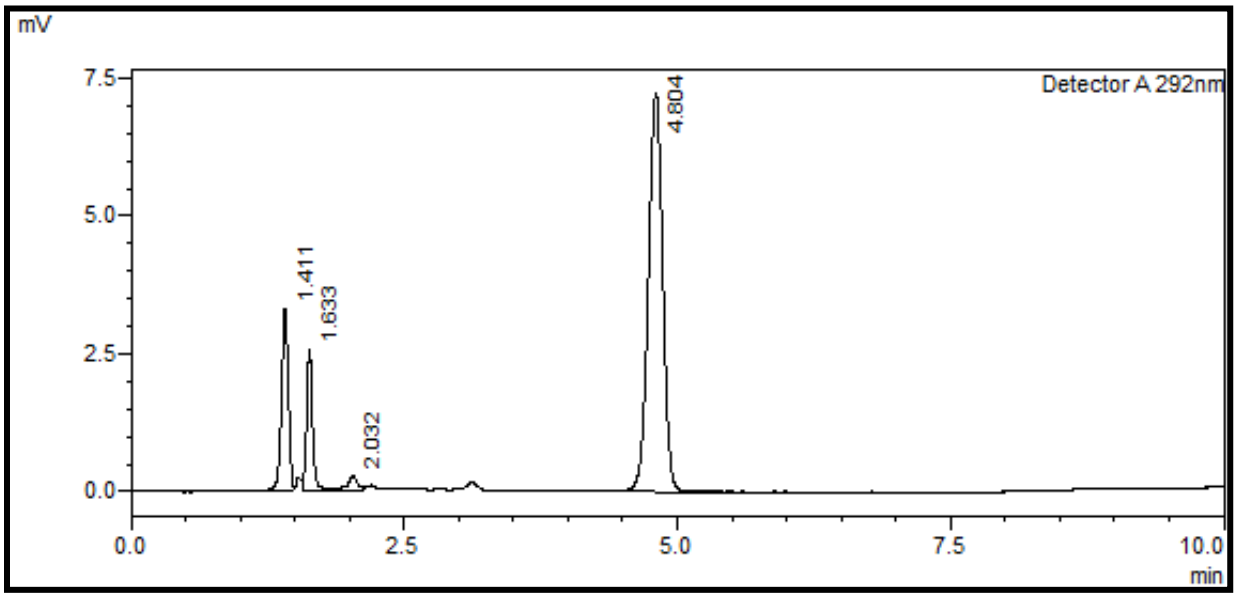

Figure S1. Method precision (ruggedness) analyst variation

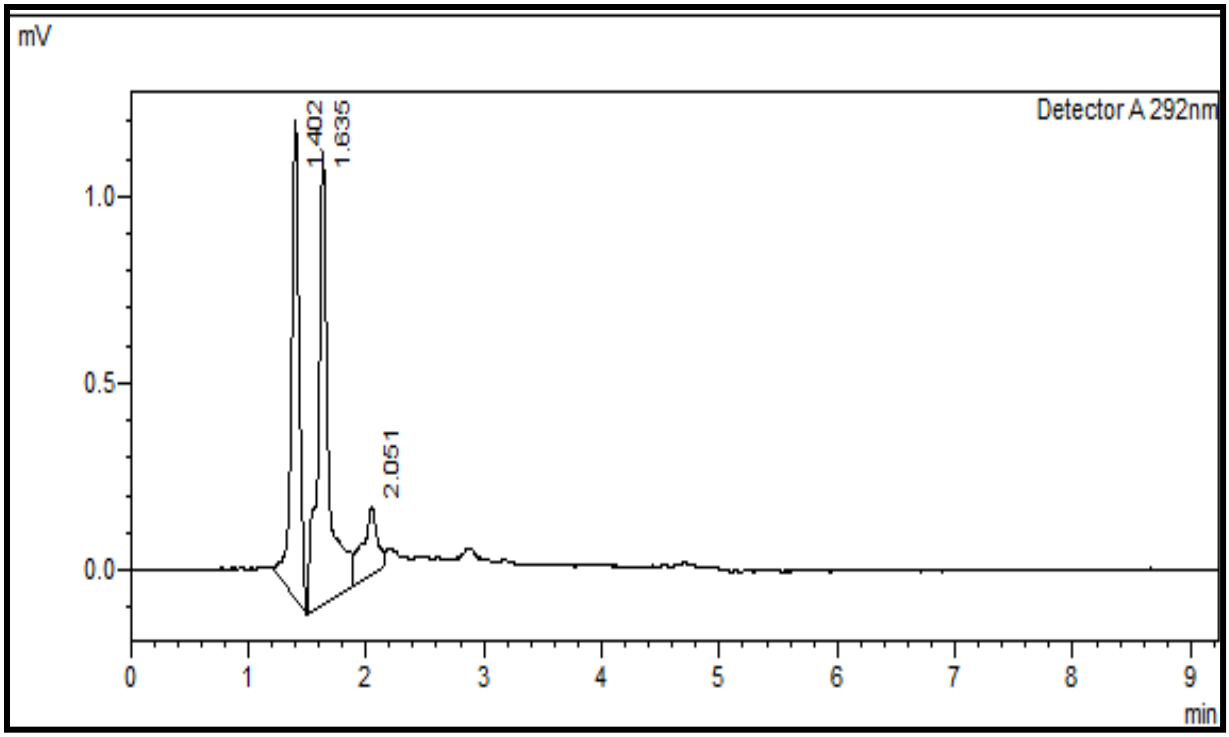

Figure S2: Specificity placebo interference.

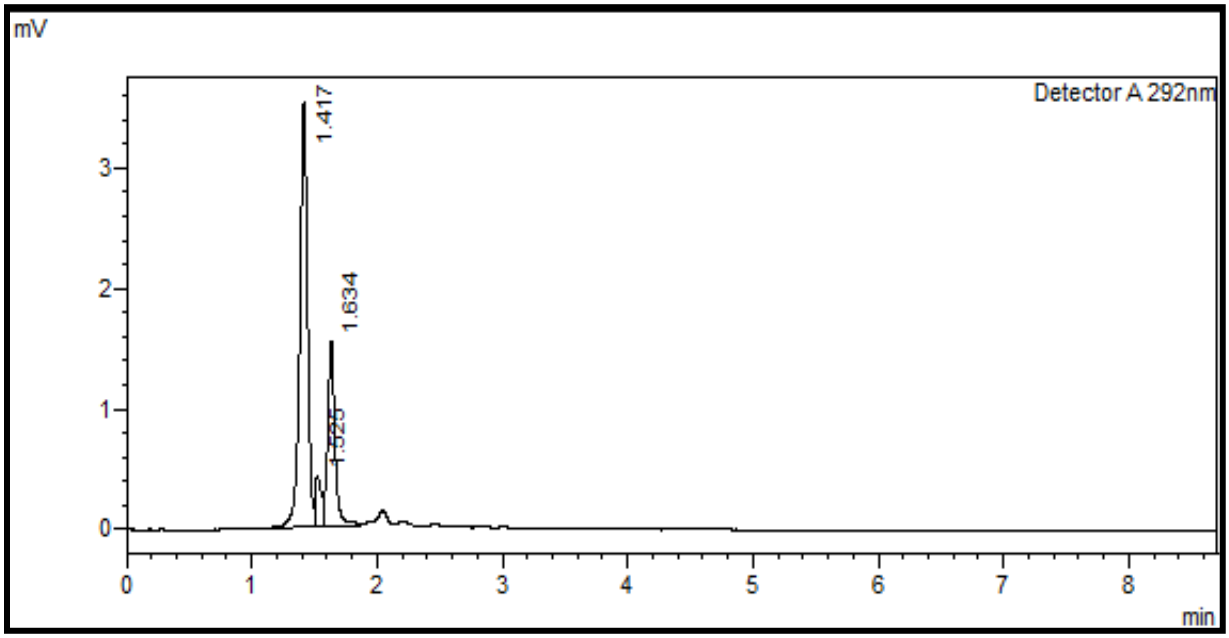

Figure S3: Specificity blank interference-diluent.

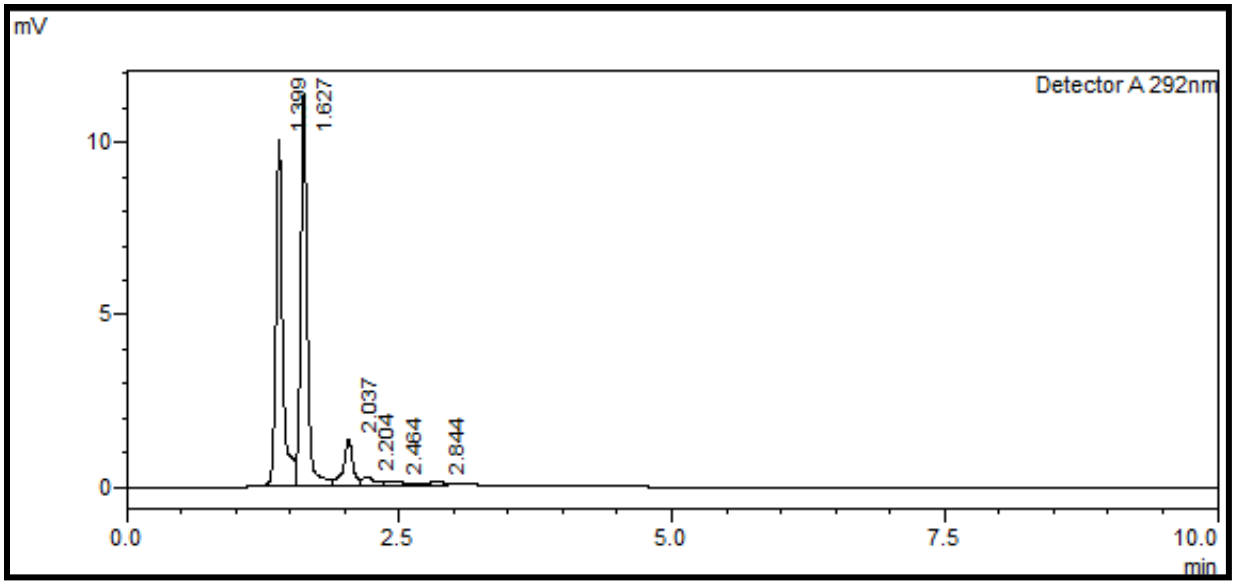

Figure S4. Specificity blank interference-mobile phase

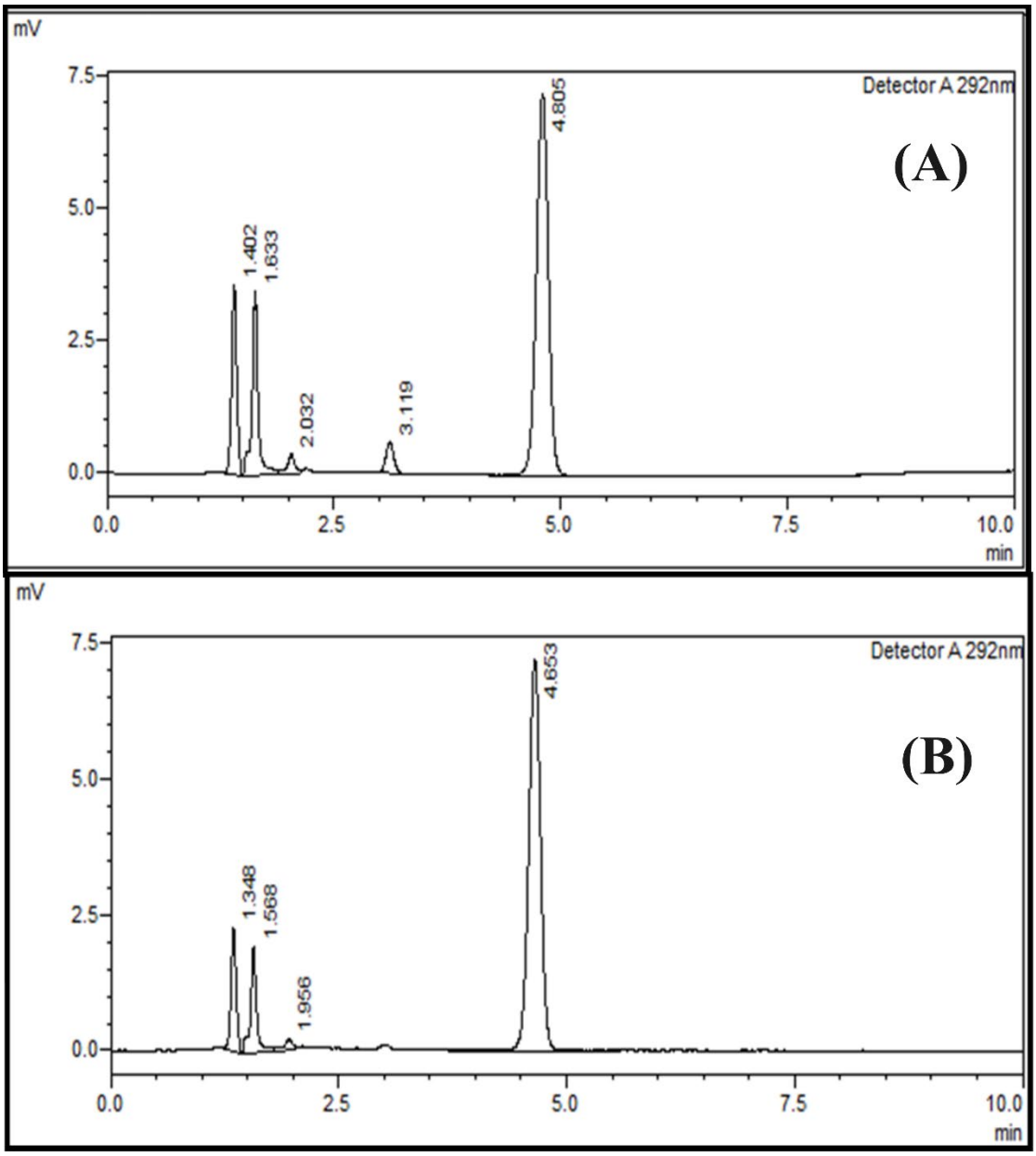

Figure S5. Method precision (robustness) at the flow rates 2.0 ml/min (A) and 2.5 ml/min (B).

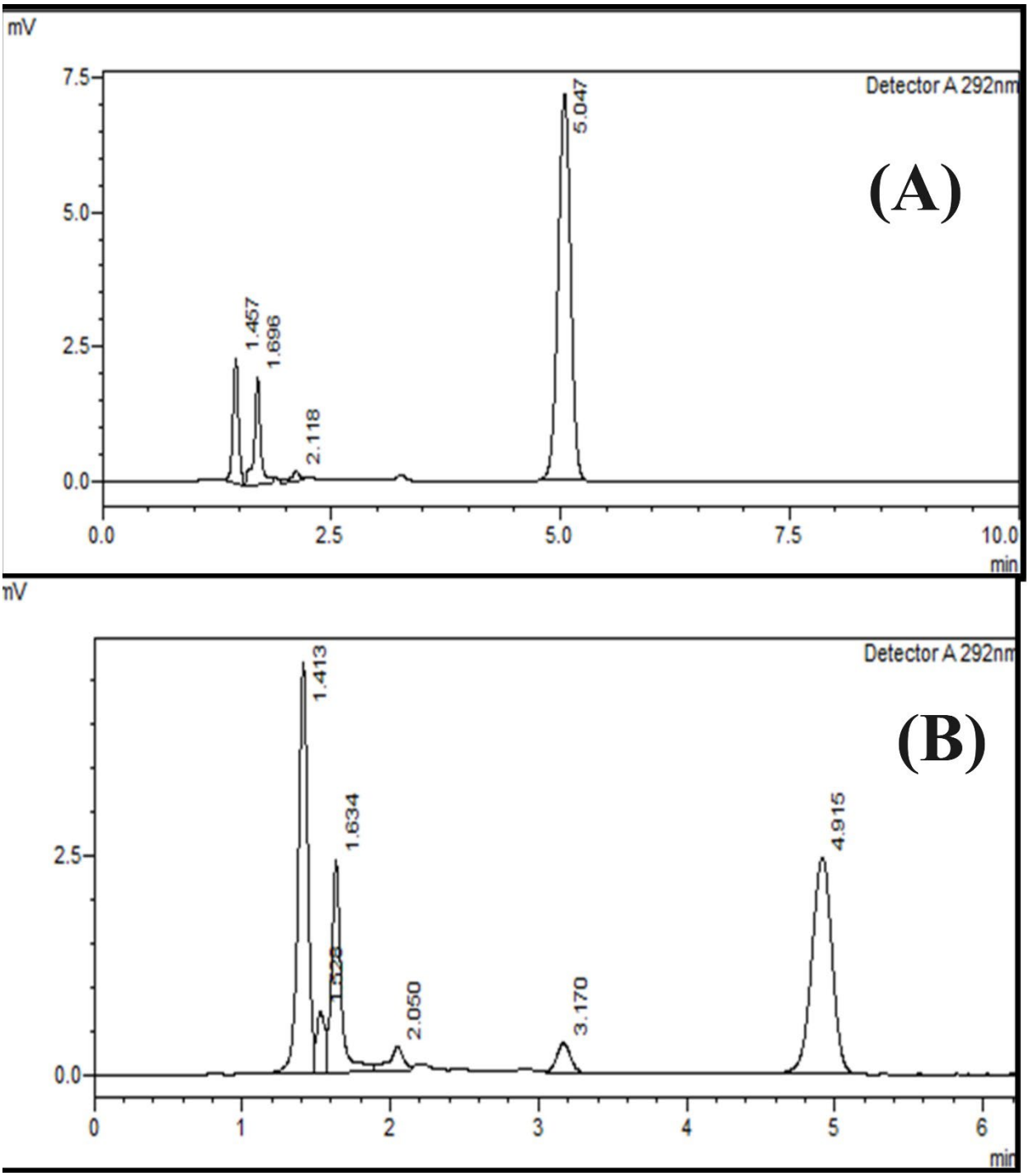

Figure S6. Robustness of the method (mobile phase (80:20) (A) and (90:10) (B).