Supplementary Files

A simultaneous extraction/derivatization strategy for quantitation of Vitamin D in Dried blood spots using LC-MS/MS: Application to biomarker study in Subjects Tested for SARS-CoV-2

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Table S1: Summary of MS/MS parameters without 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) derivatization: precursor ion, fragment ions, voltage potential (Q1), collision energy (CE) and voltage potential (Q3) for analytes.

Analyte	MRM (precursor/fragment ions)	Q1 (V)	CE (V)	Q3 (V)
25(OH)D2	413.15>395.4	-15	-11	-15
25(OH)D3	401.15>383.4	-10	-10	-15
Vitamin D2	397.3>69.1	-19	-25	-13
Vitamin D3	385.3>367.4	-18	-13	-14
d-3 vitamin D2	400.35>69.1	-19	-26	-13
d-6 25(OH)D3	407.2>389.4	-19	-11	-15

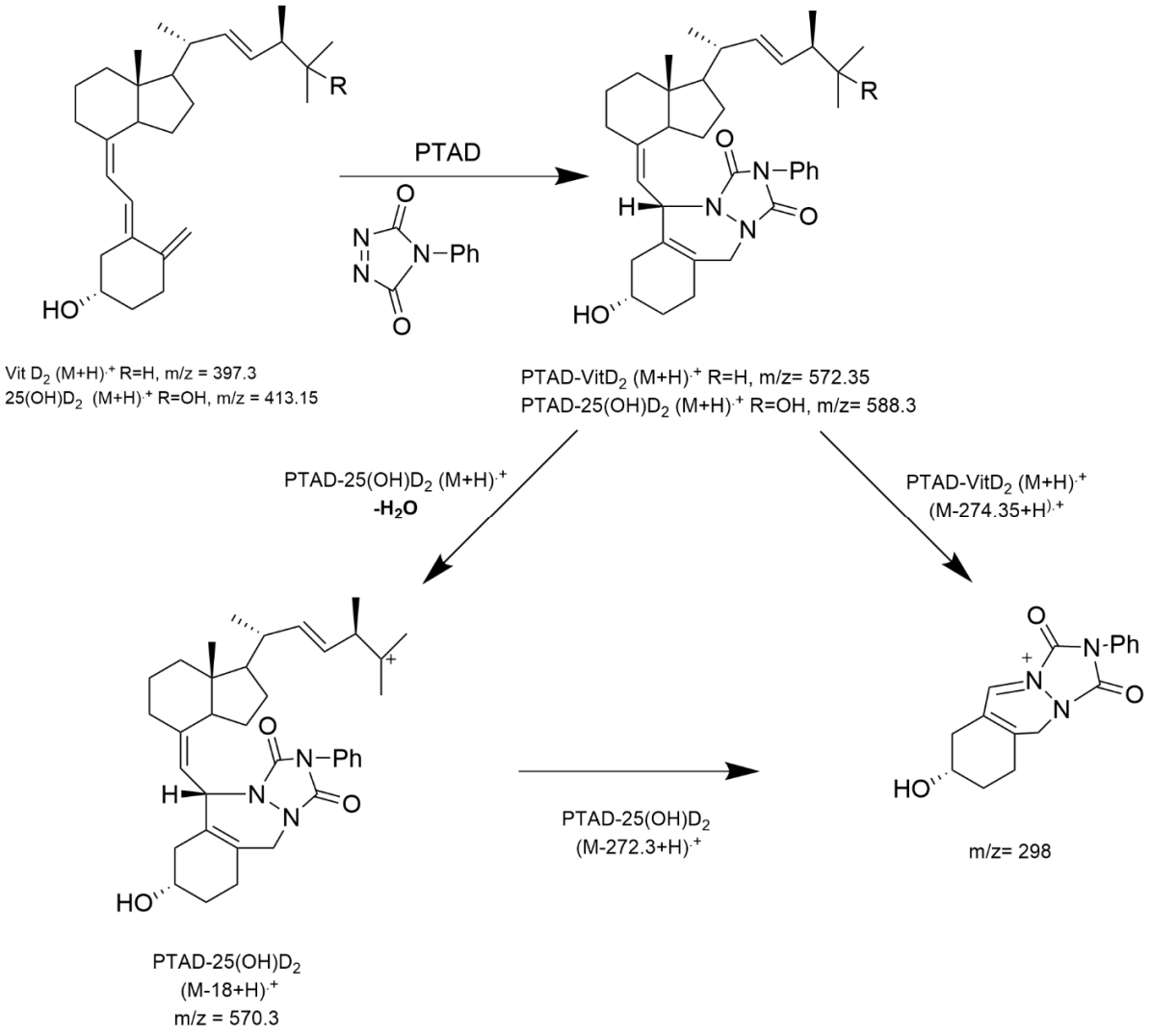

Figure S1. PTAD derivatization of vitamin D2 and 25(OH)D2 showing the PTAD-adduct ions at 572.35 and 588.3 respectively and the dehydrated precursor ions for 25(OH)D2 at 570.2.

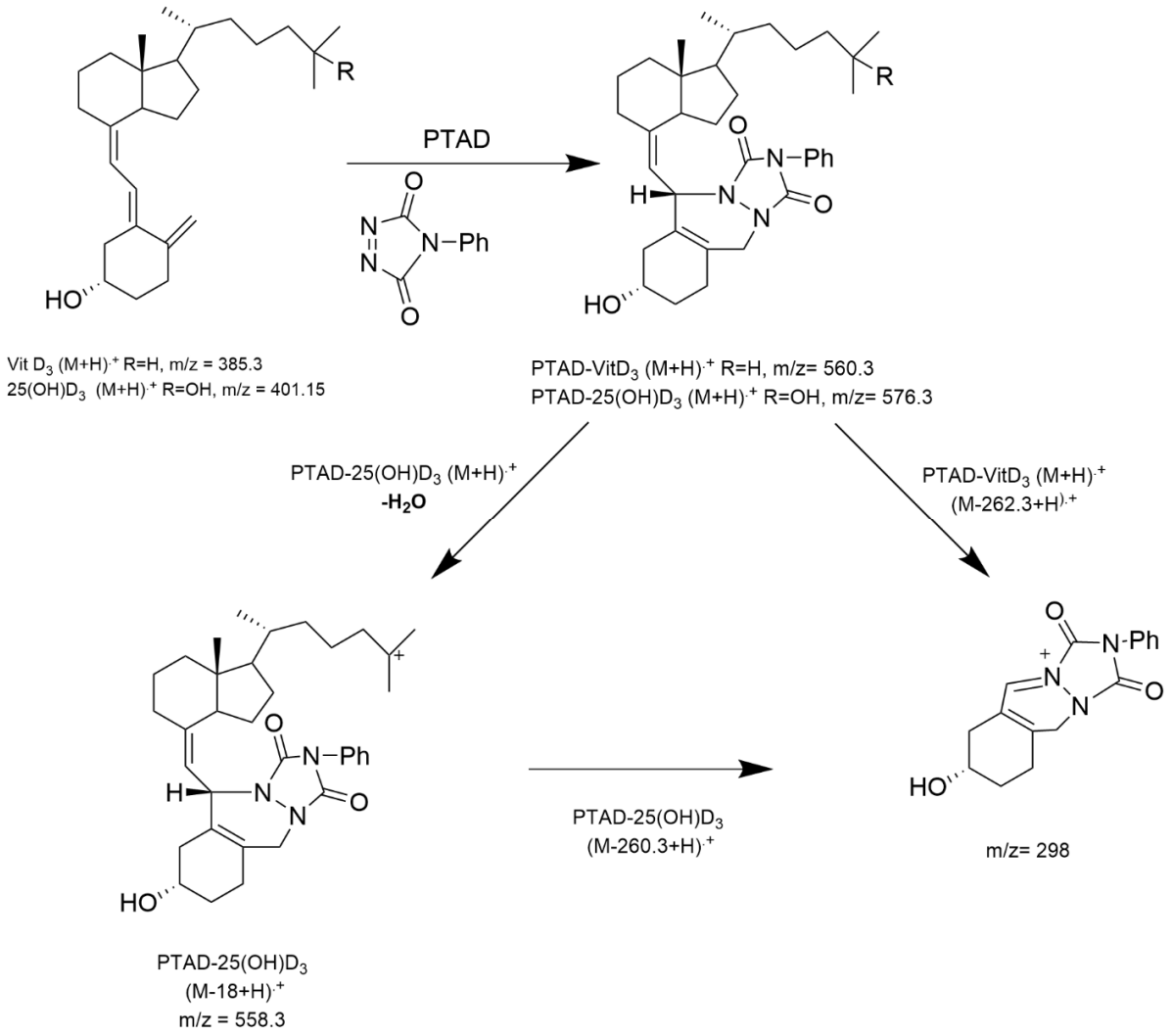

Figure S2. PTAD derivatization of vitamin D3 and 25(OH)D3 showing the PTAD-adduct ions at 560.3 and 576.3 respectively and the dehydrated precursor ions for 25(OH)D3 at 558.3.

Table S2: Summarizes the comparison of this method with previously reported LC-MS/MS methods related to the quantitation of vitamin D in Dried blood spots and human plasma.

Method	Analytes	Internal standard	Bio-matrix	Linearity (ng/mL)	Limit of detection (ng/mL)	Run time (min)	Ref.
LC-ESI-MS/MS	25(OH)D3	d-6 25(OH)D3	DBS	2.25-225	LOD: 5	10	[26]
LC-API-MS/MS	25(OH)D3 25(OH)D2	d-3 25(OH)D3 d-3 25(OH)D2	Serum	2.5-100	LLOQ: 2.5	7	[37]
LC-ESI-MS/MS	25(OH)D3 25(OH)D2	d-6 25(OH)D3	Serum	1-100	LLOQ: 1	3	[38]
LC-ESI-MS/MS (This method)	25(OH)D3 25(OH)D2 Vitamin D3 Vitamin D2	d-6 25(OH)D3 d-3 vitamin D2	DBS	0.78-200	LLOQ: 0.78	11	

References

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