

Table S1. A comparison of our method with previously reported methodologies.

Analytes	Instrument	Column	Ion source	Sample consumption	Derivatization or not	Derivatization Reagents	Derivatization time and temperature	Sample Pretreatment	Separation time	Extraction recovery	Matrix effects	Sensitivity	Reference
D ₃ , D ₂ , 25(OH)-D ₃ , 25(OH)-D ₂ , 1,25-(OH) ₂ -D ₃ , 1,25-(OH) ₂ -D ₂	UHPLC-MS/MS	C18	ESI	20 µL plasma	No	NA	NA	Protein precipitation	12 min	76.7-96.8%	100.2-103.8%	LOD: 0.02-0.1 ng/mL LOQ: 0.04-0.4 ng/mL	Method in this paper
1,25(OH) ₂ -D ₃ , 1,25(OH) ₂ -D ₂ , 25(OH)-D ₂	LC-HRMS	Phenyl-Hexyl column	APCI, DBDI, ESI	200 µL serum	No	NA	NA	Protein precipitation+SPE	20 min	Not reported	70-140%	LOD: 1-15 ng/mL	[1]
25(OH)-D ₃ , 25(OH)-D ₂	LC-MS/MS	Ascentis F5	APCI	250–1000 µL serum	No	NA	NA	LLE	12 min	91±1.4% 95±1.2%	No report	LOQ: 0.60- 1.85 ng/mL	[2]
25(OH)-D ₃ , 25(OH)-D ₂	LC- MS/MS	C8	APCI	200 µL serum/plasma	No	NA	NA	Protein precipitation+LLE	9 min	61.1-84.3%	99.7-128.9%	LOQ: 4.95-5.01 ng/mL	[3]
25(OH)-D ₃ , 25(OH)-D ₂	LC-MS/MS	C18	APCI	50 µL serum/plasma	No	NA	NA	LLE	4.5 min	82.5-101.7%	No significant matrix effect	LOQ: 13.62-20.63 ng/mL	[4]
25(OH)-D ₃ , 25(OH)-D ₂ , 24,25-(OH) ₂ -D ₃ , 1,25-(OH) ₂ -D ₃ , 1,25-(OH) ₂ -D ₂	UHPLC-MS/MS	C18	ESI	200 or 50 µL plasma	Yes	4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)	Room temperature for 1 h	SPE	4.5 min	55.6-84.9%	85-115%	LOQ: 0.01-0.02 ng/mL	[5]
25(OH)-D ₂ , 25(OH)-D ₃ , 3-epi-25(OH)-D ₃ , 1,25(OH) ₂ -D ₃ , 1,25(OH) ₂ -D ₂ , 24,25(OH) ₂ -D ₃	UHPLC- MS/MS	PFP 100 A	ESI	50 µL serum	Yes	Amplifex Diene Reagent Kit	4 °C for 30 min	SLE	17.5 min	62-74%	100±1.7%	LOQ: 0.01-1 ng/mL	[6]
25(OH)-D ₃	LC-MS/MS	T3	ESI	200 µL plasma	Yes	diene adduct	Room temperature for 30 min	Protein precipitation+LLE	15 min	No report	No report	LOQ: 6.25 ng/mL	[7]
25(OH)-D ₃	GC-MS/MS	HP-5MS	No report	200 µL plasma	Yes	trimethylsilyl	60 °C for 40 min	Protein precipitation+LLE	9 min	95-112%	No report	LOD: 1.5 ng/mL	[7]
1,25(OH) ₂ -D ₃	SIM/GC-MS	Superox 4 fused silica open tube	No report	20 mL serum	Yes	trimethylsilyl	NA	SPE	5 min	70.9%	No report	0.0025 ng/mL	[8]

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