Supplemental information for

Acylation derivatization based LC-MS analysis of vitamin D metabolites from finger-prick blood

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RUNNING TITLE: Finger-Prick Analysis of Vitamin D by Acylation

DISCLOSURE STATEMENT: The authors have nothing to disclose.

Supplemental Table S1. MRM parameters for original vitamin D metabolites and their INC derivatization products.

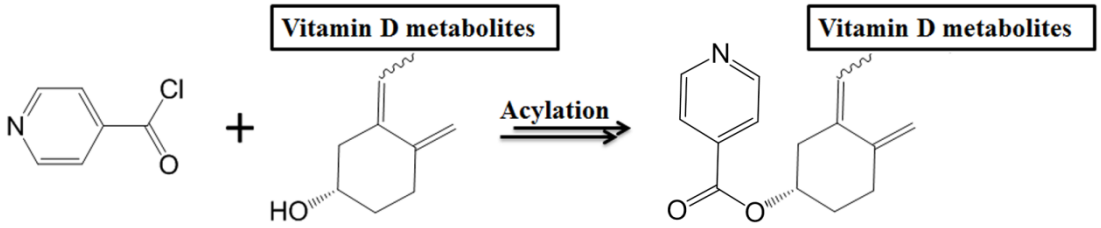
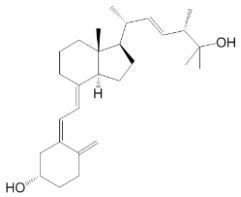
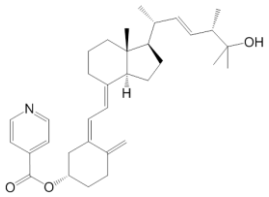
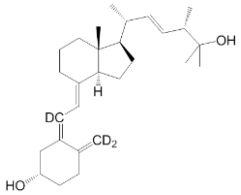
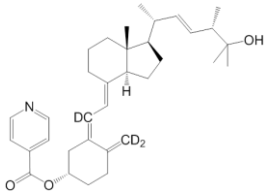
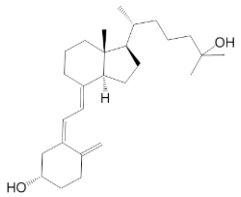
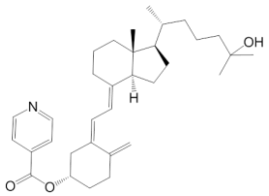
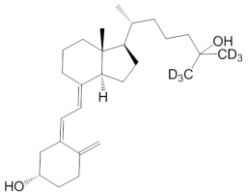
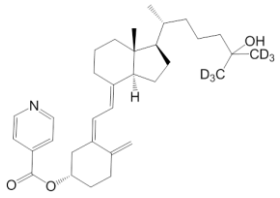
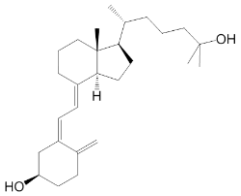
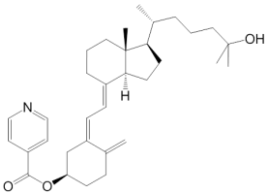
Targets	Precursor ion (m/z)	Product ion (m/z)	DP (v)	CE (eV)
$25(OH)D_2$[a]	413.2	395.4	90	13
$25(OH)D_2$[b]		159.1	90	35
$25(OH)D_2$-d3[a]	416.3	398.4	60	12
$25(OH)D_2$-d3[b]		358.5	60	13
$25(OH)D_3$[a]	401.2	365.3	100	15
epi-$25(OH)D_3$[a]				
$25(OH)D_3$[b]	401.2	383.3	100	13
epi-$25(OH)D_3$[b]				
$25(OH)D_3$-d6[a]	407.2	371.3	90	14
$25(OH)D_3$-d6[b]		389.4	90	13
$24,25(OH)_2D_2$[a]	429.4	393.4	90	12
$24,25(OH)_2D_2$[b]		411.7	90	11
$24,25(OH)_2D_3$[a]	417.4	381.4	120	17
$24,25(OH)_2D_3$[b]		399.5	120	18
$1,25(OH)_2D_2$[a]	429.3	411.4	60	9
$1,25(OH)_2D_2$[b]		393.4	60	15
$1,25(OH)_2D_3$[a]	417.4	399.4	90	9
$1,25(OH)_2D_3$[b]		381.4	100	12
$25(OH)D_2$-INC[a]	518.3	124.0	90	65
$25(OH)D_2$-INC[b]		395.4	90	28
$25(OH)D_2$-d3-INC[a]	521.4	124.0	80	70
$25(OH)D_2$-d3-INC[b]		398.3	80	25
$25(OH)D_3$-INC[a]	506.3	124.0	90	70
$25(OH)D_3$-INC[b]		365.3	90	30
epi-$25(OH)D_3$-INC[a]	506.4	124.0	90	70
epi-$25(OH)D_3$-INC[b]		365.4	90	30
$25(OH)D_3$-d6-INC[a]	512.3	124.0	90	70
$25(OH)D_3$-d6-INC[b]		371.2	90	25
$24,25(OH)_2D_2$-INC[a]	534.4	124.0	60	66
$24,25(OH)_2D_2$-INC[b]		393.2	50	20
$24,25(OH)_2D_3$-INC[a]	522.5	124.0	60	62
$24,25(OH)_2D_3$-INC[b]		381.2	50	26
$1,25(OH)_2D_2$-INC[a]	534.4	124.0	80	70
$1,25(OH)_2D_2$-INC[b]		393.3	80	24
$1,25(OH)_2D_3$-INC[a]	522.5	124.0	80	70
$1,25(OH)_2D_3$-INC[b]		399.0	60	13

Abbreviation: MRM, Multiple Reaction Monitoring; DP, Decluttering Potential; CE, Collision Energy.

[a] Quantifier ion.

[b] Qualifier ion.

Supplemental Table S2. Schematic of INC derivatization of 25(OH)D and structure information of related targets.

Schematic of INC derivatization of 25(OH)D*		
		
Target	**Intact structure**	**INC derivatized structure**
$25(OH)D_2$		
$25(OH)D_2$-d_3		
$25(OH)D_3$		
$25(OH)D_3$-d_6		
epi-$25(OH)D_3$		

* During MRM detection, the isonicotinic fragment showed best intensity for all the 25(OH)D-INC species so that the protonized isonicotinic acid ion (m/z 124.0) was chosen as product ion for quantification.

Supplemental Table S3. The calibration curves and sensitivity for the INC derivatization method.

Targets*	Retention time (min)	Linear range (ng/mL)	Calibration curves			LLOQ (ng/mL)
			Slope	Intercept	R^2	
25(OH)D_2	10.65	1.0-100.0	0.0352	-0.0096	0.9980	1.0
25(OH)D_3	10.55	1.0-100.0	0.0301	0.0390	0.9990	1.0
epi-25(OH)D_3	10.70	1.0-100.0	0.0434	0.0372	0.9970	1.0

* The targets were detected as the 25(OH)D-INC forms.

Supplemental Table S4. Intra- and inter-assay accuracy and imprecision of the the INC derivatization method.

Targets	Theoretical value (ng/mL)	Intraday (n = 6)		Interday (n = 30)	
		Measured concentration (ng/mL) ± SD	Recoveries% (CV%)	Measured concentration (ng/mL) ± SD	Recoveries% (CV%)
$25(OH)D_2$					
LLOQ-level	1.0	0.9 ± 0.2	93.5 (16.8)	0.9 ± 0.2	90.6 (17.9)
low-level	10.0	9.1 ± 0.8	90.6 (9.2)	9.2 ± 0.8	91.1 (9.3)
medium-level	30.0	28.5 ± 2.5	95.1 (8.8)	28.2 ± 2.6	94.1 (9.1)
high-level	100.0	97.1 ± 6.3	97.1 (6.5)	96.5 ± 7.8	96.5 (8.1)
$25(OH)D_3$					
LLOQ-level	1.0	0.9 ± 0.1	88.8 (14.1)	0.9 ± 0.1	87.4 (14.8)
low-level	10.0	9.2 ± 0.6	92.3 (6.2)	9.0 ± 0.7	89.9 (8.1)
medium-level	30.0	27.6 ± 2.0	92.1 (7.1)	28.0 ± 2.3	93.2 (8.3)
high-level	100.0	100.7 ± 5.4	100.7 (5.4)	95.5 ± 7.1	95.5 (7.4)
epi-$25(OH)D_3$					
LLOQ-level	1.0	0.9 ± 0.1	86.5 (12.1)	1.0 ± 0.1	102.5 (11.5)
low-level	10.0	9.7 ± 0.7	97.2 (6.8)	9.6 ± 0.8	96.4 (8.7)
medium-level	30.0	31.1 ± 1.9	103.6 (6.1)	28.7 ± 2.8	95.6 (9.6)
high-level	100.0	95.4 ± 6.1	95.4 (6.4)	93.6 ± 6.5	93.6 (6.9)

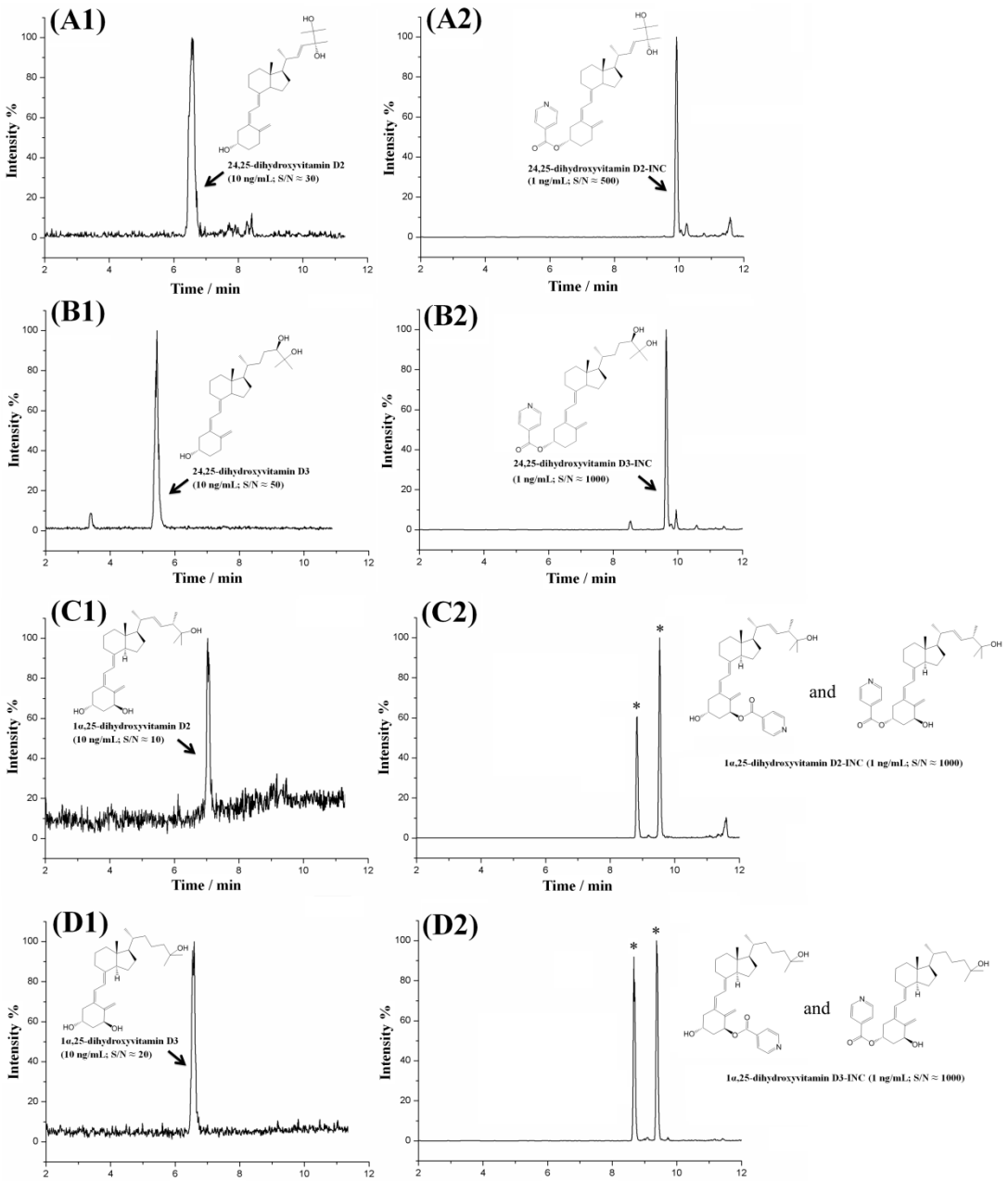
Supplemental Table S5. Certified and measured concentrations of $25(OH)D_2$, $25(OH)D_3$ and epi-$25(OH)D_3$ from the reference material SRM927a.

SRM	Targets	Certified value (ng/mL)	Measured value (n=5, ng/mL)	Recoveries (%)	CV (%)
927a	$25(OH)D_2$				
	level 1	0.5[b]	-[c]	-	-
	level 2	0.8[a]	-[c]	-	-
	level 3	13.3[a]	12.5 ±0.9	94.2	7.5
	level 4	0.6[b]	-[c]	-	-
	$25(OH)D_3$				
	level 1	28.8[a]	27.5 ±2.0	95.6	7.3
	level 2	18.1[a]	16.9 ±1.1	93.5	6.4
	level 3	19.8[a]	18.6 ±1.5	94.1	8.1
	level 4	29.4[a]	27.8 ±1.9	94.4	7.0
	epi-$25(OH)D_3$				
	level 1	1.8[a]	1.6 ±0.2	90.5	10.1
	level 2	1.3[a]	1.1 ±0.1	86.3	12.5
	level 3	1.2[b]	1.0 ±0.1	84.1	10.2
	level 4	26.0[a]	24.9 ±1.8	95.6	7.1

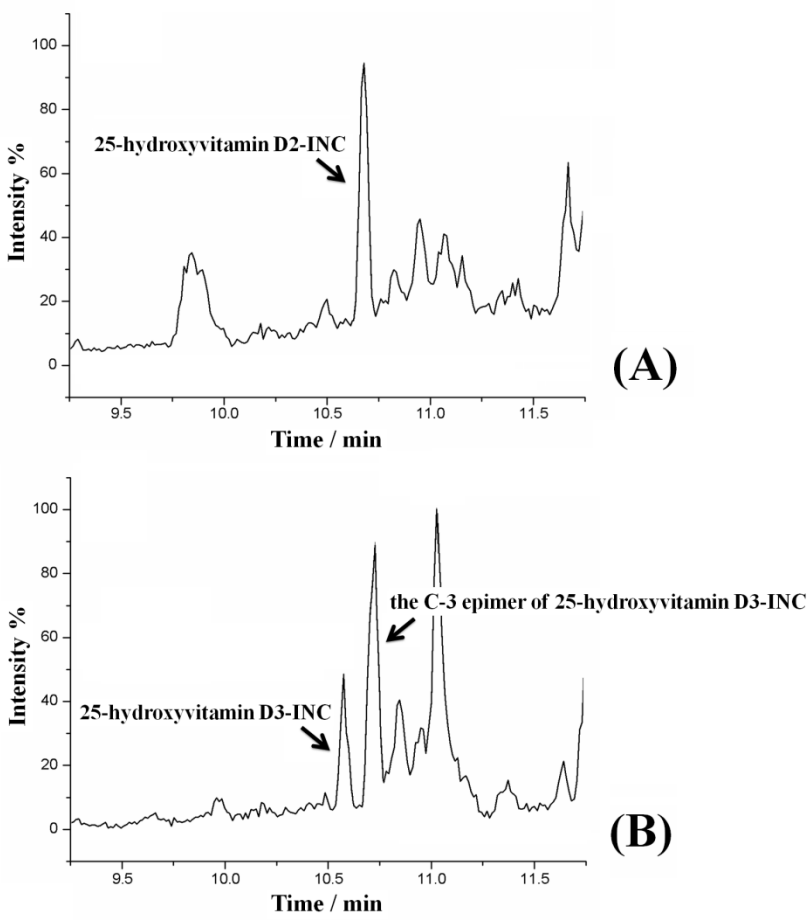
[a] Certified value.

[b] Reference value.

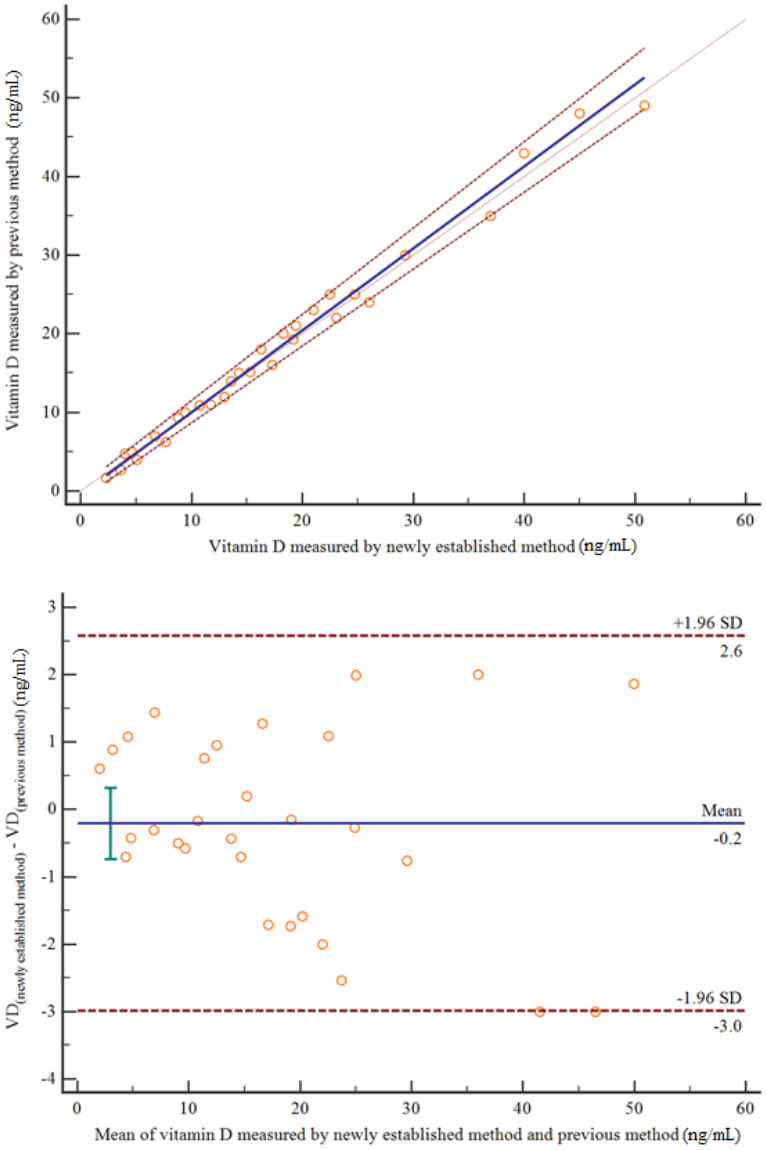
[c] Lower than LLOQ.



Supplemental Figure S1. The typical LC-MS/MS chromatograms of 24,25$(OH)_2D_2$, 24,25$(OH)_2D_3$, 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$ before (A1, B1, C1 and D1, 10 ng/mL) and after (A2, B2, C2 and D2, 1 ng/mL) INC derivatization.



Supplemental Figure S2. The typical LC-MS/MS chromatograms of 25(OH)D-INC in spiked plasma sample on LLOQ-level, (A) 25(OH)D_2-INC; (B) 25(OH)D_3-INC and epi-25(OH)D_3-INC.



Supplemental Figure S3. Comparison of vitamin D values measured by newly established method and previous method in 30 samples (plasma). (A) Passing-Bablok regress analysis for newly established method and previous method. (B) Bland-Altman plot showed the bias between the newly established method and previous method.

Investigation of reaction yields

Plasma (100 μL) from high-level of QC samples was transferred into a polypropylene conical centrifuge tube. After mixing with ACN (200 μL), dichloromethane (900 μL) was added, vortexed (1 min) and centrifuged (3000 g, 30 s) for liquid-liquid extraction (LLE). The resulted subnatant (900 μL) was then transferred into a new polypropylene conical centrifuge tube, dried under nitrogen gas and redissolved in a mixture (200 μL) of ethyl acetate and dichloromethane (1/3, v/v). Such mixture was then divided into two parts (each 100 μL). For Part-I, INC solution (10 μL) was added for derivatization. For Part-II, ACN (10 μL) was added for control. The final mixture was then dried under nitrogen gas again and reconstituted with initial mobile phase (100 μL) for LC-MS/MS analysis of 25(OH)D species. The reaction yields could be evaluated by comparing the results from Part-I to Part-II.