Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

for

Determination of vitamin D metabolites in various biological samples through an improved chemical derivatization assisted liquid chromatography-tandem mass spectrometry

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The Supporting information includes following items:

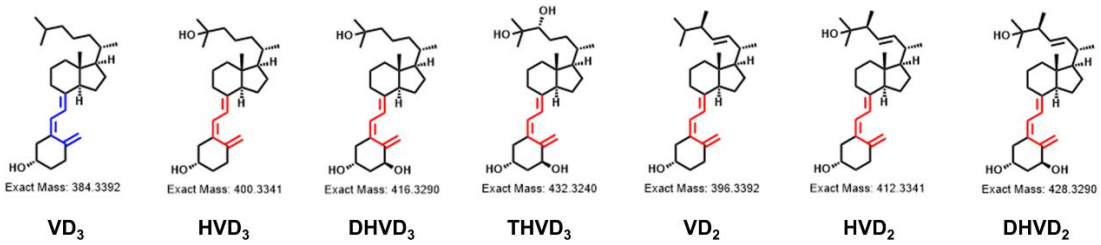

Fig. S1. The chemical structures of 7 VD metabolites.

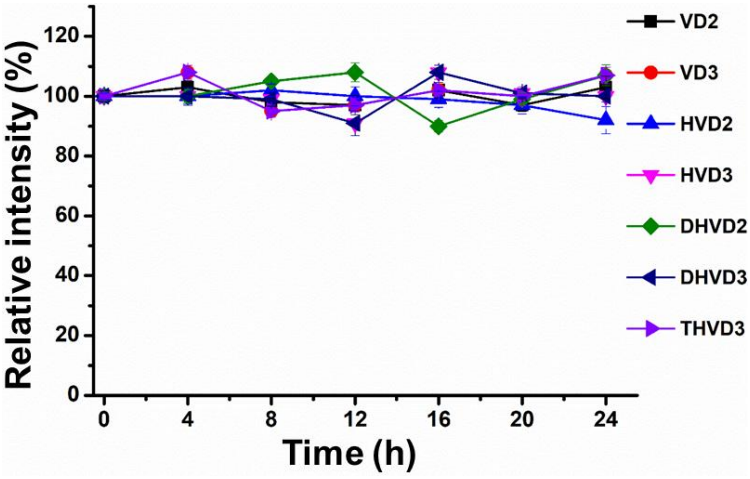

Fig. S2. Stability of 7 PTAD derivatives under 4 °C for 24 h.

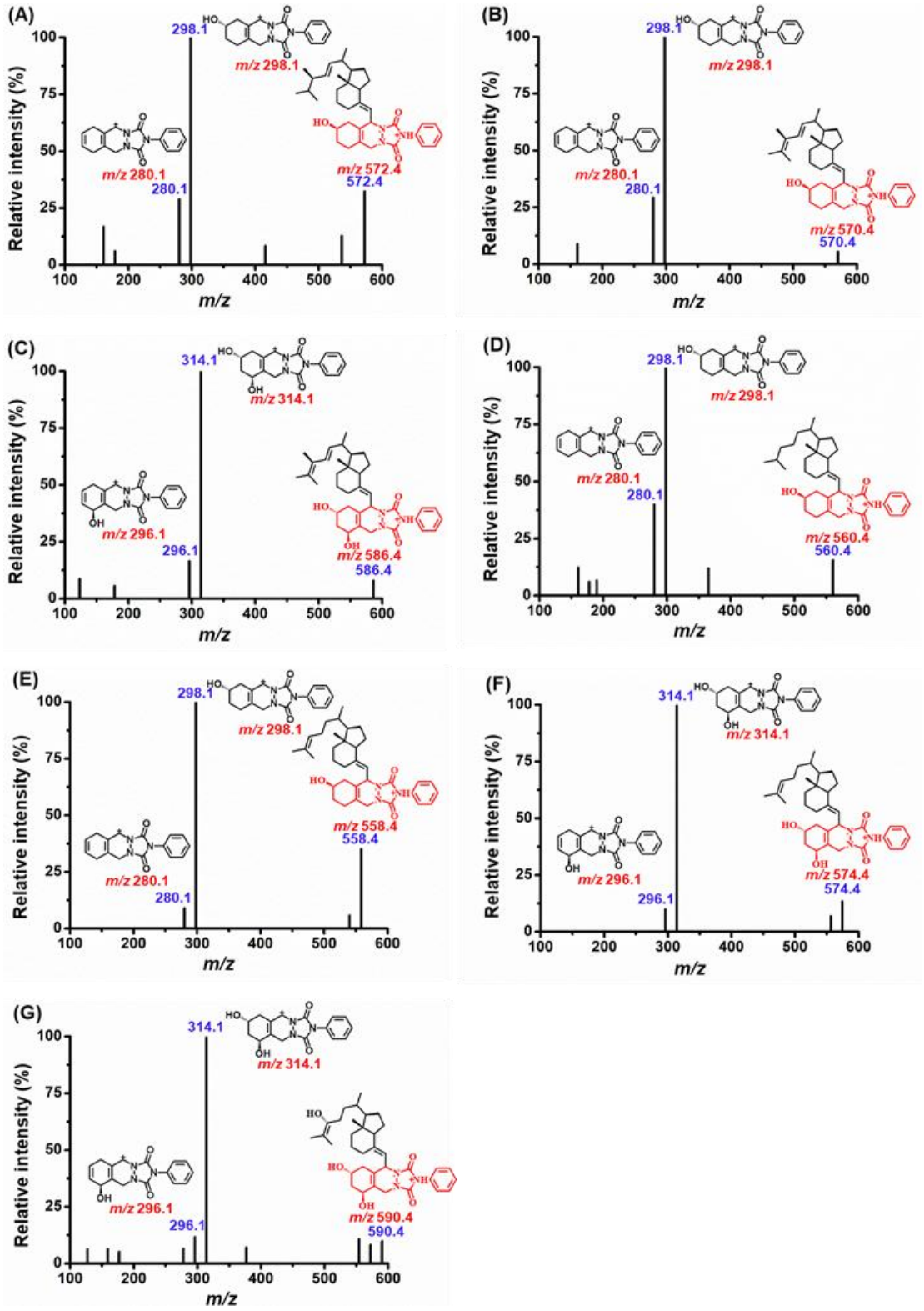

Fig. S3. MS/MS spectra of 7 VD metabolites after PTAD derivatization. (a) VD_2. (b) VD_2. (c) $DHVD_2$. (d) VD_3. (e) HVD_3. (f) $DHVD_3$. (g) $THVD_3$.

Table S1. MRM transition parameters of PTAD-derived VDs.

Analytes	Scan mode	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
VD_2	+	572.3	**298.1**	50	-26	19	-30
			280.1	50	-26	29	-29
HVD_2	+	570.3	**298.1**	50	-28	18	-30
			280.1	50	-26	30	-29
$DHVD_2$	+	586.3	**314.1**	50	-26	21	-14
			296.1	50	-26	31	-30
VD_3	+	560.3	**298.1**	50	-20	18	-20
			280.1	50	-26	30	-29
HVD_3	+	558.3	**298.1**	50	-26	16	-20
			280.1	50	-26	28	-29
$DHVD_3$	+	574.3	**314.1**	50	-20	18	-14
			296.1	50	-20	28	-19
$THVD_3$	+	590.3	**314.1**	50	-28	23	-14
			296.1	50	-26	30	-30
VD_2-d_3	+	575.3	**301.1**	50	-20	21	-20
			283.1	50	-20	30	-30
$DHVD_3$-d_3	+	577.3	**317.1**	50	-20	22	-22
			299.1	50	-20	30	-20

The product ions in **bold** were used for quantification.

Table S2. PTAD derivatization efficiency of 7 VDs in standard solution, human serum, mouse liver, and cell extract.

Analytes	Derivatization efficiency (%)			
	Standard solution	Serum extract	Liver extract	Cell extract
VD_3	99.7	98.1	92.1	88.9
VD_2	99.0	110.8	72.3	95.6
HVD_3	97.0	94.4	85.6	103.1
HVD_2	99.7	86.3	75.3	88.6
$DHVD_3$	92.5	79.6	111.2	90.3
$DHVD_2$	97.0	103.1	94.4	84.7
$THVD_3$	96.0	86.0	70.6	106.8

Table S3. LODs, LOQs, linear dynamic range and regression curve of PTAD-derived VDs.

Analytes	LODs (pg mL^{-1})	LOQs (pg mL^{-1})	Linear dynamic range (ng mL^{-1})	Regression line		
				Slope	Intercept	R^2
VD_3	3	10	0.05-50	0.23	0.10	0.9963
VD_2	3	10	0.05-50	0.23	0.19	0.9936
HVD_3	10	30	0.10-100	0.25	0.22	0.9962
HVD_2	7	20	0.10-100	0.29	0.33	0.9968
$DHVD_3$	13	40	0.10-100	0.31	0.35	0.9943
$DHVD_2$	10	30	0.10-100	0.07	0.08	0.9933
$THVD_3$	20	70	0.20-200	0.12	0.23	0.9966

Table S4. Precisions (intra- and inter-day) and recoveries for analysis of PTAD-derived VDs in three different concentrations (0.2, 2, and 50 ng mL^{-1}).

Analytes	Intra-day precision (RSD, %; n = 5)			Inter-day precision (RSD, %; n = 3)			Recoveries (%; n = 3)		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
VD_3	14.2	7.6	5.8	9.7	7.3	6.1	79.1	89.8	97.6
VD_2	12.1	6.3	3.4	14.5	6.8	5.8	87.9	98.4	102.3
HVD_3	7.5	7.5	4.4	16.5	9.2	7.9	78.8	93.6	110.2
HVD_2	9.7	6.1	3.7	15.6	8.4	5.5	108.4	89.2	92.1
$DHVD_3$	15.5	5.7	3.5	10.4	8.7	6.2	92.5	85.6	96.8
$DHVD_2$	7.4	7.3	3.2	14.9	9.7	5.7	84.7	103.1	102.2
$THVD_3$	8.4	8.3	4.5	9.2	7.6	7.1	85.7	100.5	100.2

Table S5. Extraction efficiency and matrix effect for VDs from human serum, mouse liver, and MLF cell using our established method.

Analytes	Extraction efficiency (%)			Matrix effect (%)		
	Serum	Liver	Cell	Serum	Liver	Cell
VD_3	81.3	87.5	88.4	81.3	108.2	91.3
VD_2	86.9	86.0	86.3	86.9	75.2	106.9
HVD_3	100.3	70.4	85.9	100.3	98.6	103.8
HVD_2	75.5	75.9	86.2	75.5	82.6	75.7
$DHVD_3$	79.0	80.8	87.2	79.0	79.9	119.1
$DHVD_2$	98.8	92.8	104.9	98.8	77.8	98.8
$THVD_3$	83.9	89.6	83.5	73.9	83.0	83.9