

**Table I.**

Method validation and stability data for free and total analyses

	Free		Total			
	HMB	DHB	HMB	DHB	THB	2,5-DHMB
Matrix concentration range (ng/mL)	15–150	15–150	200–2,000	40–400	200–2,000	40–400
LOD (ng/mL)	0.648	6.22	68.7	1.62	81.9	11.0
LLOQ <sup>a</sup> (ng/mL)	15	15	200	40	200	40
Accuracy (RE %)						
Intra-day <sup>b</sup>	≤ ± 5.4	≤ ± 6.9	≤ ± 10.1	≤ ± 5.6	≤ ± 5.0	≤ ± 8.1
Inter-day <sup>b</sup>	≤ ± 3.4	≤ ± 3.2	≤ ± 7.0	≤ ± 3.4	≤ ± 2.4	≤ ± 4.2
Intra-day <sup>c</sup>	≤ ± 8.1	≤ ± 13.8	≤ ± 4.9	≤ ± 12.8	≤ ± 17.5	≤ ± 8.2
Inter-day <sup>c</sup>	≤ ± 6.3	≤ ± 11.0	≤ ± -3.1	≤ ± 3.9	≤ ± 11.1	≤ ± 2.5
Precision RSD (%)						
Intra-day <sup>b</sup>	≤10.8	≤27.2	≤29.6	≤2.7	≤9.0	≤12.5
Inter-day <sup>b</sup>	≤5.0	≤14.4	≤14.4	≤3.0	≤4.9	≤4.2
Intra-day <sup>c</sup>	≤3.9	≤10.8	≤11.6	≤28.5	≤4.5	≤8.6
Inter-day <sup>c</sup>	≤3.9	≤8.1	≤10.4	≤16.3	≤10.2	≤7.4

	Free		Total			
	HMB	DHB	HMB	DHB	THB	2,5-DHMB
Stability (RE %)						
Freeze–thaw matrix	–3.0 to 1.7	–8.2 to 5.0	–1.1 to 0.0	0.3 to 3.5	NS	NS
Short-term matrix (RT)	–3.5 to 1.7	–4.8 to 7.5	1.3 to 1.9	–2.4 to –0.2	NS	NS
Long-term matrix (–70°C)	–6.0 to 5.9	–1.3 to 13.6	2.3 to 2.5	3.5 to 6.4	–9.8 to –8.5	11.0 to 18.8
Extended matrix (–70°C)	ND	ND	–5.7 to 18.3	–4.5 to 2.7	–36.5 to –22.2	–20.5 to –79.0
Extract 2–8 s°C	ND	ND	≤123.3	≤52.3	≤63.6	≤60.5
Recovery %						
Absolute	213	117	80 <sup>c</sup>	71.1 <sup>c</sup>	62.9 <sup>c</sup>	65.1 <sup>c</sup>
Relative	95.6	88.4	92.3	91.6	93.2	91.0
Reinjection reproducibility (RE%)	≤4.1	≤4.1	≤13	≤6.3	≤2.4	≤5.7

<sup>a</sup>Lower limit of quantitation (LLOQ) is the lowest concentration of the calibration curve. ND, not determined; NS, not stable.

<sup>b</sup>Estimated based on plasma calibration standards.

<sup>c</sup>Estimated based on low, mid and high QC samples.