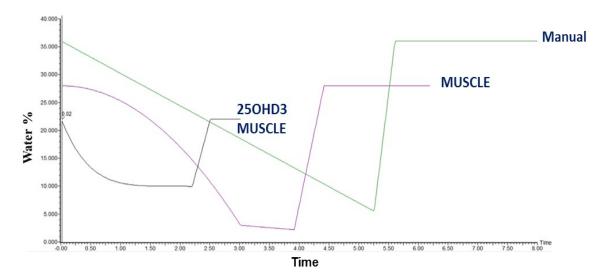
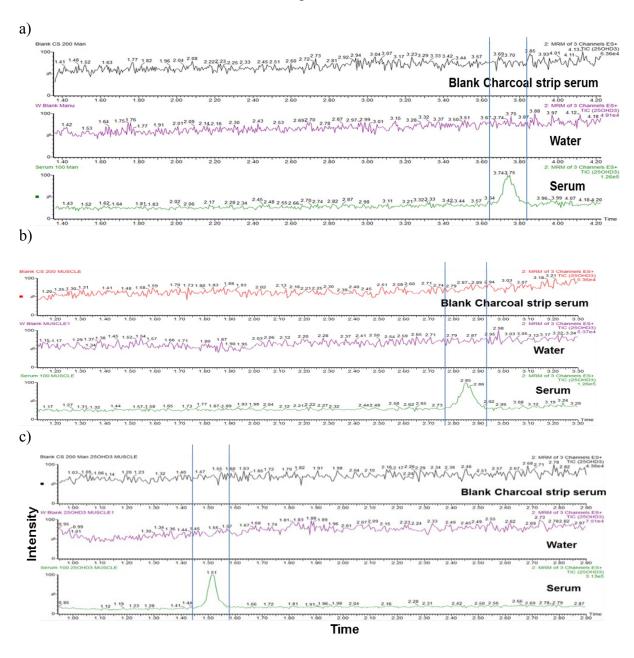
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Supporting information Figure 1 Overlaid inlet gradients comparing; green line – manual developed method, purple line – optimised MUSCLE method, and black line – 25OHD3 MUSCLE method



**Supporting information Figure 2** Post column infusion of 25OHD3 for a) manual developed method, b) MUSCLE method and c) 25OHD3 MUSCLE method. The concentration of infused 25OHD3 was 16 ng/mL



**Supporting information Figure 3** Bland Altman plots for a) 25OHD3 comparison with manual and MUSCLE methods, b) 3-Epi-25OHD3 comparison between manual and MUSCLE methods, c)  $24,25(OH)_2D3$  with the MUSCLE method and manual method and d) 25OHD2 with the MUSCLE method and manual method, showing the mean bias (solid line)  $\pm$ SD (dotted lines)

