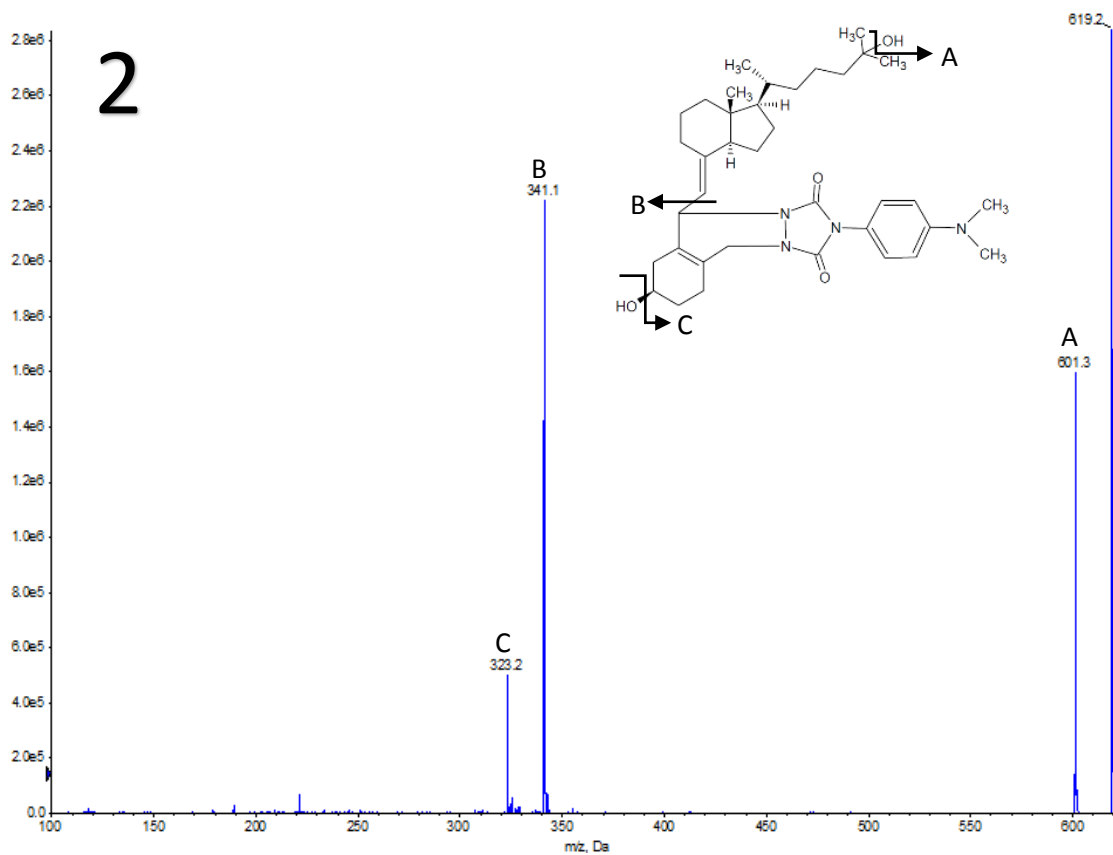
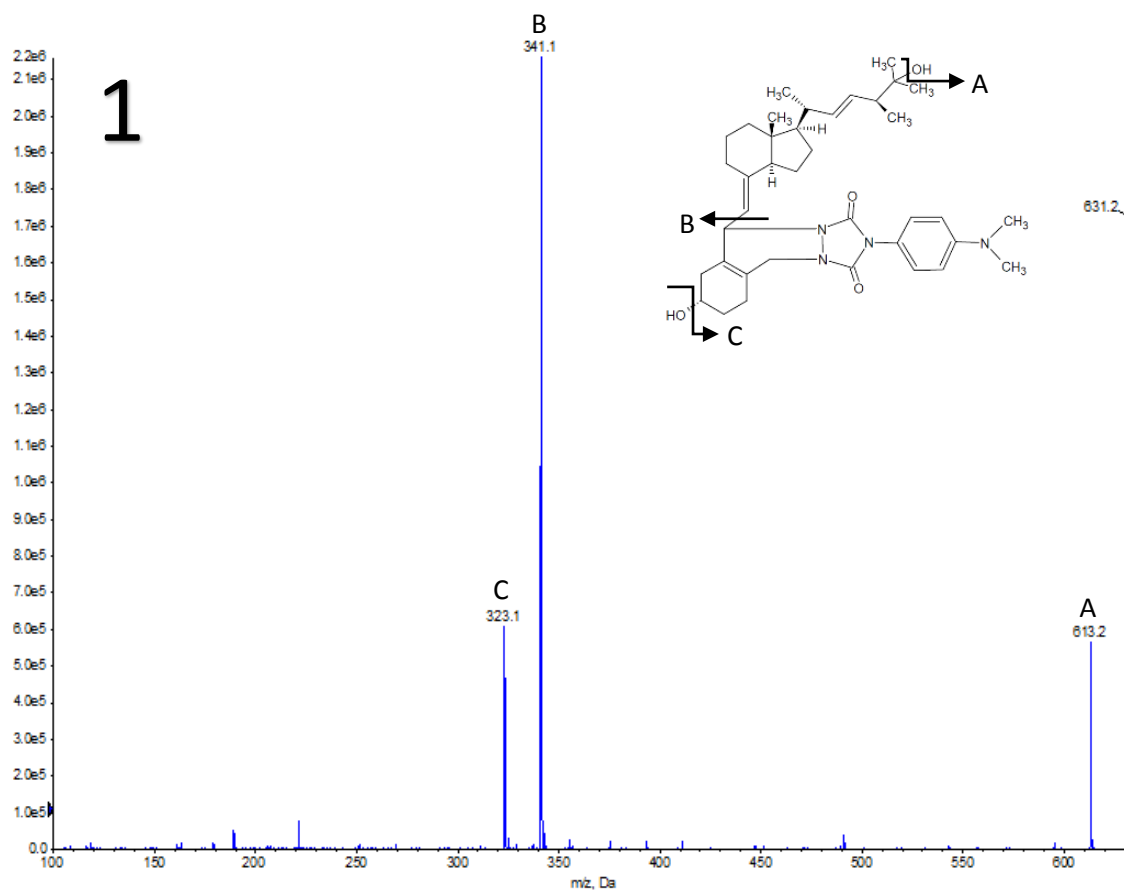
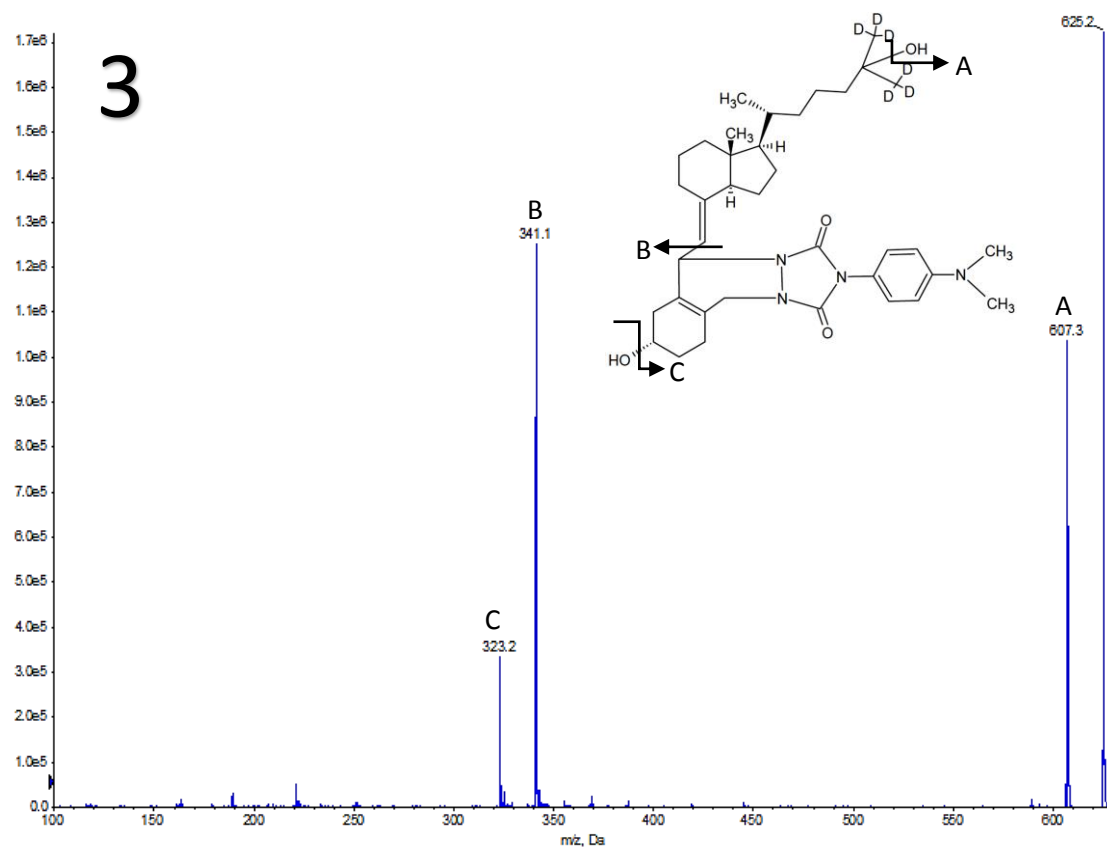
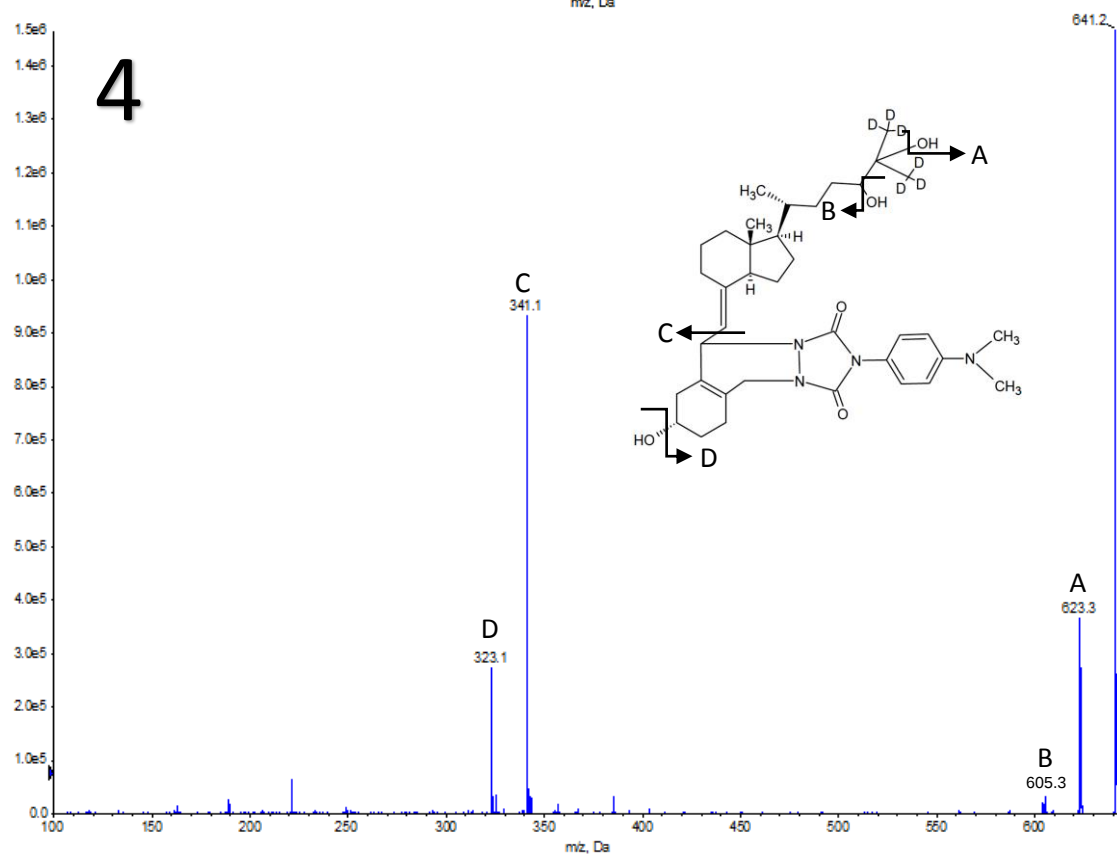




3



4



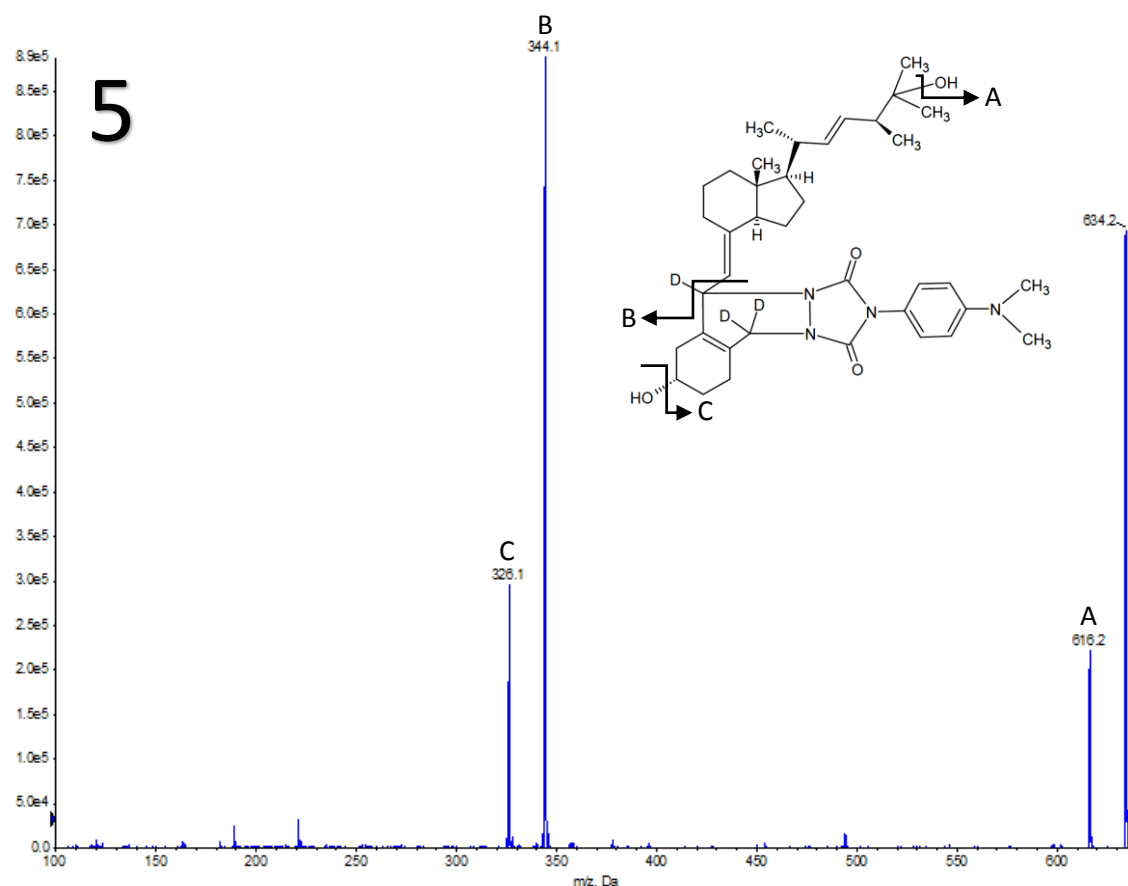


Figure 1S. Fragment spectra of studied compounds with the identification of daughter ions. Operation parameters of MS/MS system: curtain gas (CUR) 30, collision gas (CAD) 7, ionspray voltage (IS) 3500 V, temperature of drying gas (TEM) 500°C, ion source gas 1 (GS1) 40, ion source 2 (GS2) 50, declustering potential (DP) 40 V, entrance potential (EP) 10 V, collision energy (CE) 30 V, collision cell exit potential (CXP) 13 V. Notation: 1 - 25(OH)D2, 2 - 3-epi-25(OH)D3, 3 - d6-25(OH)D3, 4 - d6-24,25(OH)2D3, 5 - d3-25(OH)D2.

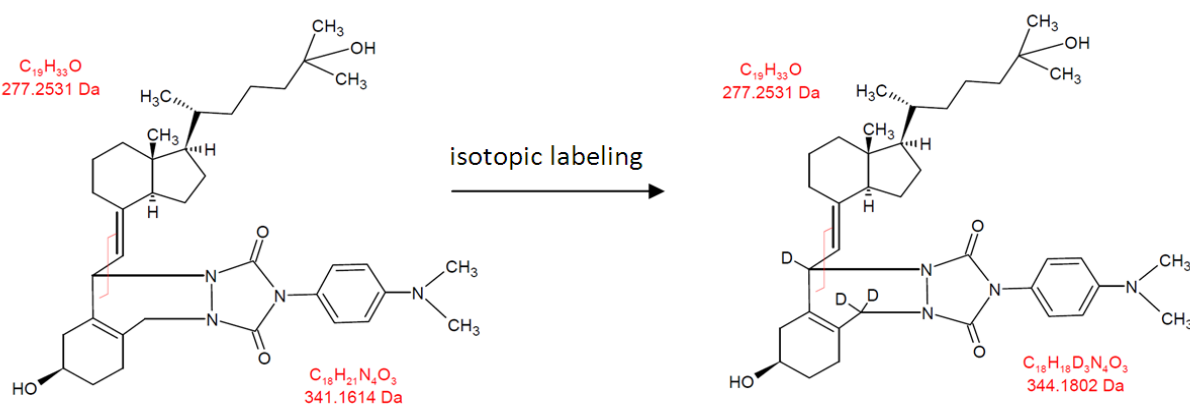


Figure 2S. The effect of substituting hydrogen atoms with deuterium atoms on the mass of a characteristic fragment ion.

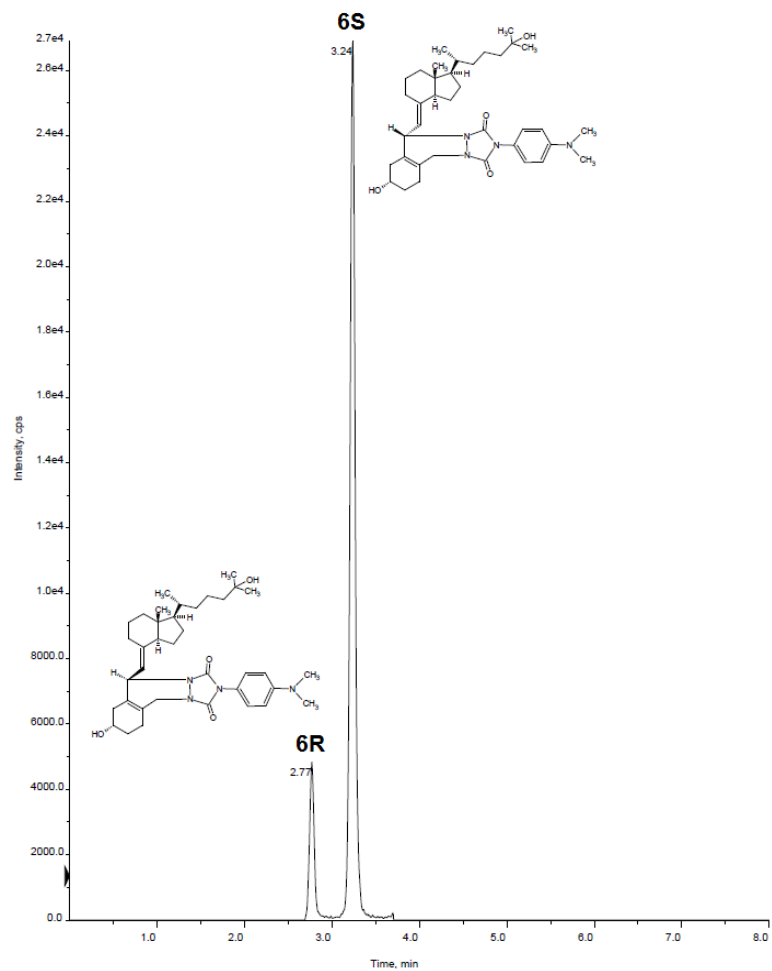


Figure 3S. Chromatogram of 24,25(OH)₂D₃ with assigned isomers. Conditions: Kinetex F5 (1.7 μ m; 50 x 2.1 mm); mobile phase: water (A) and acetonitrile (B) with 0.1% FA; flow rate: 0.45 ml min⁻¹; gradient elution program: 0 min. – 30%B, 5.5 min. – 50%B, 5.7 min. – 95%B, 6.6 min. – 95%B, 6.8 min. – 30%B, 8 min. – 30%B; column oven temperature: 40°C.

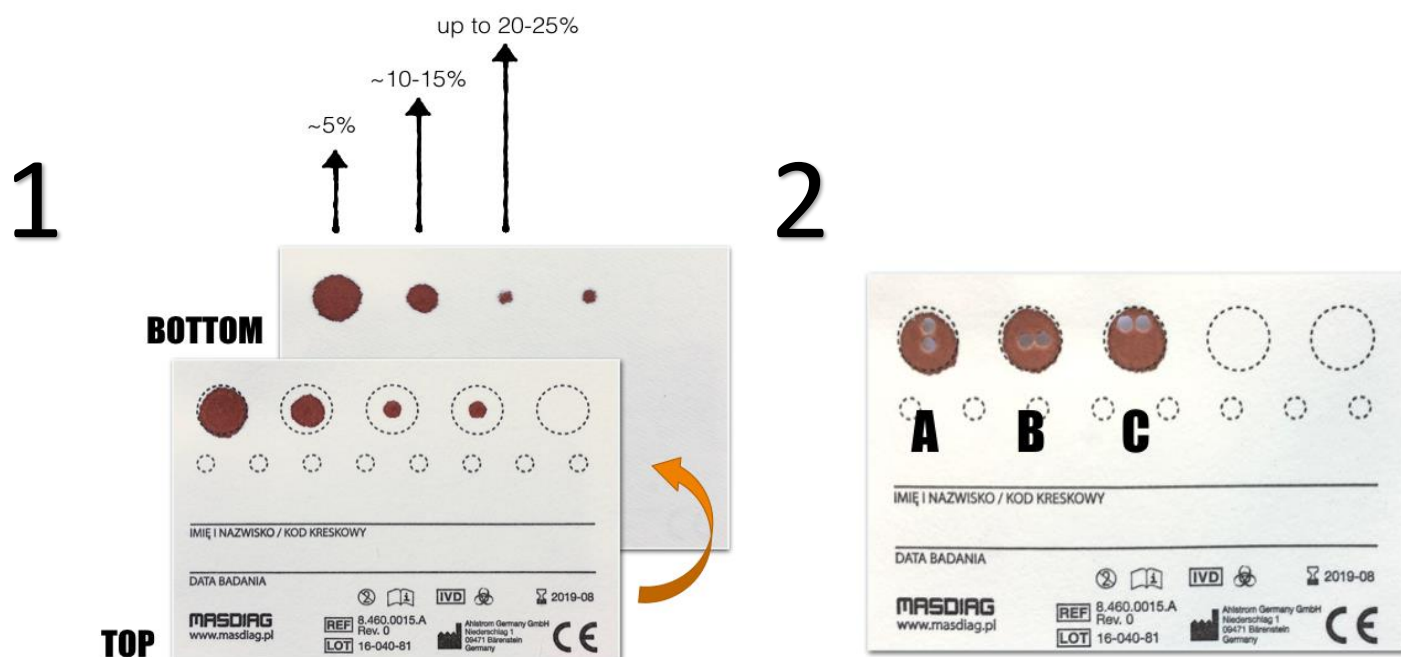


Figure 4S. Different volumes of blood applied to the paper and the corresponding bias from the actual value (1). Various possibilities of cutting discs (2). Notation: A - one in the center, one on the edge; B - two in the center; C - two on the edges.

Table 1S. Results for between-day assay imprecision depending on the storage conditions.

	25(OH)D	24,25(OH) ₂ D ₃	3-epi-25(OH)D ₃
room temperature, desiccator, sunlight			
Average [ng ml ⁻¹]	58.7	6.29	2.60
SD	2.75	0.34	0.21
CV [%]	4.68	5.39	7.89
room temperature, desiccator, without sunlight			
Average [ng ml ⁻¹]	57.42	6.28	2.58
SD	3.72	0.42	0.23
CV [%]	6.47	6.65	9.01
room temperature, without desiccator, sunlight			
Average [ng ml ⁻¹]	56.28	6.13	2.43
SD	4.38	0.53	0.19
CV [%]	7.78	8.56	7.86
room temperature, without desiccator, without sunlight			
Average [ng ml ⁻¹]	57.92	6.36	2.53
SD	2.87	0.28	0.19
CV [%]	4.95	4.43	7.40
4°C, desiccator			
Average [ng ml ⁻¹]	61.25	6.56	2.63
SD	4.40	0.45	0.16
CV [%]	7.19	6.86	6.26
4°C, without desiccator			
Average [ng ml ⁻¹]	57.92	6.37	2.57
SD	2.49	0.32	0.21
CV [%]	4.30	4.97	8.13
-18°C, desiccator			
Average [ng ml ⁻¹]	61.42	6.59	2.74
SD	3.81	0.51	0.15
CV [%]	6.21	7.71	5.59