

Figure S8. MS³ spectra acquired in analyses of control (A-E) and SPG5 (F) plasma samples shown in chromatograms displayed in Figure 8. The spectra confirm the presence of dihydroxy-3-oxocholest-4-en-26-oic acids in plasma. (A) CA⁴-7 α ,24-diol-3-one, (B & C) *syn* and *anti* conformers of CA⁴-7 α ,x-diol-3-one, and (D & E) *syn* and *anti* conformers of CA⁴-7 α ,12 α -diol-3-one from control plasma, (F) CA⁴-7 α ,x-diol-3-one in SPG5 plasma. Data was acquired in the LIT analyser of the Orbitrap Elite hybrid instrument with an accuracy of m/z \pm 0.1 for most fragment-ions.

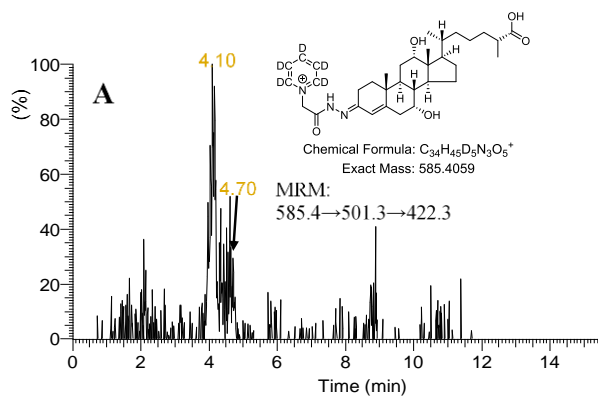


Figure S9. LC-MS³ MRM chromatogram highlighting the CA⁴-7 α ,12 α -diol-3-one structure in plasma from CTX patient. The corresponding LC-HRMS chromatogram is shown in Figure 8C. Data was acquired in the LIT analyser of the Orbitrap Elite hybrid instrument with an m/z window of ± 0.4 . Retention times were correlated to a QC sample analysed within the same batch of samples.

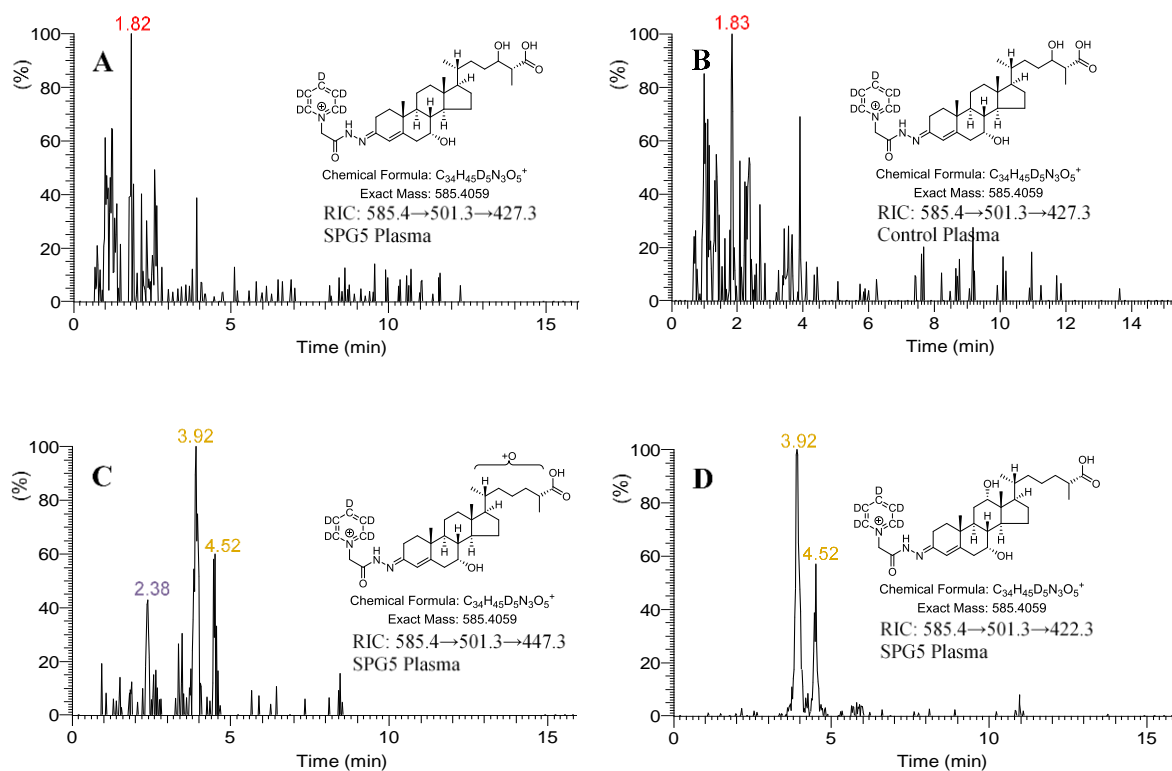


Figure S10. LC-MS³ MRM chromatograms highlighting (A & B) CA⁴-7 α ,24-diol-3-one, (C) CA⁴-7 α ,x-diol-3-one, and (D) CA⁴-7 α ,12 α -diol-3-one. (A, C & D) are from the analysis of SPG5 plasma and (B) is from the analysis of a QC sample. The corresponding LC-HRMS chromatogram for SPG5 plasma is shown in Figure 8D. Data was acquired in the LIT analyser of the Orbitrap Elite hybrid instrument with an m/z window of ± 0.4 . Retention times were correlated to a QC sample analysed within the same batch of samples.