**Supplementary Table S1. Sterols and steroids identified in human plasma (in the oxysterol fraction SPE1-Fr-1).**

| Sterol/steroid | Oxidised/derivatised sterol/steroid | $[M]^+$ (*m/z)* | Retention time/min (relative retention time)[a] | Literature relative retention time[a]; Sterol/steroid | Relative abundance[b] | Literature value[c] ng/mL [reference] |
|---|---|---|---|---|---|---|
| Dehydrocholesterol[d] $C^{5,x}$-3β-ol | $C^{4,x}$-3-one 3-GP | 516.3948 | 12.71 (0.98) | 0.90; Desmosterol | In SPE1-Fr-2 | 500±300 [S1] |
| | | | | 0.94; 7-Dehydrocholesterol | | 40 – 140 [S2] |
| Cholesterol[d] $C^5$-3β-ol | $C^4$-3-one 3-GP | 518.4105 | 12.95 (1.00) | 1.00; Cholesterol | In SPE1-Fr-2 | 2 x $10^6$ [S3] |
| 24-Oxocholesterol[e] $C^5$-3β-ol-24-one | $C^4$-3,24-dione 3-GP | 532.3898 | 7.96 (0.61) | 0.59; 24-Oxocholesterol | 02 – 05 | NA |
| 6β-Hydroxycholesterol[f] $C^5$-3β,6β-diol | $C^4$-6β-ol-3-one 3-GP | 534.4054 | 10.71 (0.83) | 0.83; 6β-Hydroxycholesterol | 12 – 17 | NA |
| 7α-Hydroxycholesterol $C^5$-3β,7α-diol | $C^4$-7α-ol-3-one 3-GP | 534.4054 | 10.25 (0.79) | 0.80; 7α-Hydroxycholesterol | 02 – 08 | 43±48 [34]<br>44 [35] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | 13 - 63 [38] |
| 27-Hydroxycholesterol<br>$C^5$-3β,27-diol | $C^4$-27-ol-3-one 3-GP | 534.4054 | 8.26 (0.64) | 0.61; 27-Hydroxycholesterol | 100 - 100 | 154±43 [34]<br>159 [35]<br>120 ±30 [36]<br>181±52[g] [37]<br>139±35[h] [37] |
| 24S-Hydroxycholesterol<br>$C^5$-3β,24S-diol | $C^4$-24S-ol-3-one 3-GP | 534.4054 | 7.77 (0.60)<br>8.01 (0.62) | 0.59; 24S-Hydroxycholesterol<br>0.61; 24S-Hydroxycholesterol | 74 – 81 | 64±24 [34]<br>83 [35]<br>64±14 [36]<br>57±13[g] [37]<br>63±13[h] [37] |
| 3β-Hydroxycholesta-5,24-dien-27-oic acid[i,p]<br>$CA^{5,24}$-3β-ol | $CA^{4,24}$-3-one 3-GP | 546.3690 | 7.86 (0.61) | NA; 3β-Hydroxycholesta-5,24-dien-27-oic acid | 19 – 51 | NA |
| 3β-Hydroxycholest-5-en-27-oic acid[p]<br>$CA^5$-3β-ol | $CA^4$-3-one 3-GP | 548.3847 | 8.08 (0.62) | 0.62; 3β-Hydroxycholest-5-en-27-oic acid | 163 - 227 | 118 [35]<br>67±28 [39]<br>75 [40] |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7α,27-Dihydroxycholesterol<br>$C^5$-3β,7α,27-triol | $C^4$-7α,27-diol-3-one 3-GP | 550.4003 | 6.72 (0.52) | 0.49: 7α,27-Dihydroxycholesterol | 11 – 22 | NA |
| 3β,5β-Dihydroxy-B-norcholestane-6β-carboxyaldehyde<br>Aldol[j] | Aldol 6-GP[k] | 552.4160 | 10.24 (0.79) | 0.79: Aldol | 07 – 18 | <4 [41] |
| 3β,7α-Dihydroxycholest-5-en-27-oic acid[p]<br>$CA^5$-3β,7α-diol | $CA^4$-7α-ol-3-one 3-GP | 564.3796 | 6.55 (0.51)<br>7.20 (0.56) | NA; 3β,7α-Dihydroxycholest-5-en-27-oic acid | 97 - 224 | 39±26 [39]<br>(121[c]) [39]<br>24 [40]<br>(52[c]) [40] |
| Androstan-3-ol-17-one 3-glucuronide[L,m,p]<br>A-3-ol-17-one 3-GlcA | A-3-ol-17-one 3-GlcA 17-GP[k] | 600.3279 | 1.38 (0.11) | NA: Etiocholanolone 3-glucuronide[m] | 118 - 577 | 51.7±7.5 [49] |
| Androstan-3-ol-17-one 3-sulphate[L,m,p]<br>A-3-ol-17-one 3-sulphate | A-3-ol-17-one 3-sulphate 17-GP[k] | 504.2527 | 0.82 (0.06) | NA: Epiandrosterone 3-sulphate[m] | 148 - 936[m,n] | [50] |
| | | | 1.22 (0.09) | NA: Androsterone 3-sulphate[m] | 577 - 955[m,n] | [50] |
| Dehydroepiandrosterone | $A^5$-3β-ol-17-one 3- | 502.2370 | 0.76 (0.06) | NA: Dehydroepiandrosterone 3- | 3140 - 3970[n] | 1501 [46] |

| | | | | | | |
|---|---|---|---|---|---|---|
| (DHEA) 3-sulphate[L,p]<br>$A^5$-3β-ol-17-one 3-sulphate | sulphate 17-GP[k] | | | sulphate | | 1030±626 [47]<br>1950 – 3312 [48] |

[a] Retention time in min, relative retention time on a scale with $C^4$-3-one 3-GP being 1.00. Literature relative retention times from reference 24.

[b] Abundance relative to $C^4$-27-ol-3-one 3-GP (100). Abundance based on ion count.

[c] Values in parenthesis represent combined values for the 3β-ol-5-ene and 3-oxo-4-ene sterols in plasma.

[d] Found predominantly in SPE1-Fr-2.

[e] $MS^n$ data indicates the oxo group is on the C-17 side-chain, retention time data suggests C-24.

[f] This may be an artefact from the oxidation/derivatisation of C-3β,5α,6β-triol.

[g] Data for males.

[h] Data for females.

[i] $MS^n$ data suggests the additional unsaturation is in the C-17 side-chain, probably at C-24.

[j] The 6-oxo group reacts with GP reagent.

[k] Not oxidised. The cholesterol oxidase enzyme from *Streptomyces* has poor activity towards $C_{19}$ and $C_{21}$ steroids. To oxidise these steroids the enzyme from *Brevibacterium* is recommended.

[L] The 17-oxo group reacts with the GP reagent.

[m] Etiocholanolone 3-glucuronide, androsterone 3-sulphate and epiandrosterone 3-sulphate are known constituents of human plasma[49,50]. The order of elution of the two 3-sulphates isomers has not been established, but it is likely that the epi isomer should be eluted first.

[n] Experimental value provides an underestimation on account of “in-source” fragmentation. See supplementary Results.

[p] Bile acids and acidic sterol and steroid conjugates (sulphates and glucronides) can be analysed without oxidation/derivatisation by negative-ion ESI.