

**Figure 1:** Chromatograms of a reaction extract from PolyCYP483 vs UCL-BT-A (821). From top to bottom the chromatograms are UV300nm, EIC302m/z (UCL-BT-A), EIC316m/z (+14Da), EIC318m/z (+16Da), EIC320m/z (+18Da) and EIC334m/z (+32Da). UCL-BT-A elutes at 1.44 minutes. An expansion of the region between 0.95 and 1.40 minutes is provided in Figure 2 below.



**Figure 2:** Expansion of the chromatograms above between 0.95 and 1.40 minutes. The UV and ESI MS spectra of the peaks at 1.03, 1.06, 1.27, 1.34 and 1.36 minutes are provides in Figures 3 to 7 below.







**Figure 3:** UV, positive ion and negative ion ESI MS spectra of the metabolite eluting at 1.03 minutes from a PolyCYP483 vs UCL-BT-A extract.







**Figure 4:** UV, positive ion and negative ion ESI MS spectra of the metabolite eluting at 1.06 minutes from a PolyCYP483 vs UCL-BT-A extract.





**Figure 5:** UV, positive ion and negative ion ESI MS spectra of the metabolite eluting at 1.28 minutes from a PolyCYP483 vs UCL-BT-A extract. This metabolite did not ionise under standard negative ion conditions.





**Figure 6:** UV, positive ion and negative ion ESI MS spectra of the metabolite eluting at 1.34 minutes from a PolyCYP483 vs UCL-BT-A extract. This metabolite did not ionise under standard negative ion conditions.





**Figure 7:** UV, positive ion and negative ion ESI MS spectra of the metabolite eluting at 1.36 minutes from a PolyCYP483 vs UCL-BT-A extract. This metabolite did not ionise under standard negative ion conditions.

**Chromatography details**

**System:**

*Pumps and autosampler:* Waters Acquity UPLC QSM and Waters Acquity UPLC FTN

*Detection (UV):* Waters Acquity UPLC PDA (UV-Vis detection)

*Detection (MS):* Waters Acquity UPLC QDA

**Basic LC gradient:**

*Column:* Waters BEH C18 1.7 μm, 2.1 mm i.d. x 50 mm length

*Column temperature:* 45°C

*Solvents:* A: 10mM ammonium bicarbonate in H2O/Acetonitrile (95/5), B: Acetonitrile

*Gradient (A%/B%):* t=0 mins: 98/2 to 2/98 at t=2.4 mins and held for a further 0.4 mins (2.8 mins total), return to 98/2 over 0.05 mins and re-equilibrated for 0.15 mins (t=3 mins) at a flow-rate of 1.0 mL/min.