

Fig. 1. Representative graphs displaying the direct quantitation of glycocholic acid and glycochenodeoxycholic acid (y axes) secreted from 2 batches of iPSC-derived hepatocytes (obtained following 2 different 2D protocols adapted from the method originally published by Sullivan and co-authors (6)) (x axes); media is used as a negative control and primary human hepatocytes (PHH) are used as a positive control.

Fig. 2. Representative graphs displaying the isotopic interference produced by the amount of glycocholic acid (GCA) or glycochenodeoxycholic acid (GCDCA), contained into the media, on the quantitation of $^{13}\text{C}_3$ - glycocholic acid ($^{13}\text{C}_3$ -GCA) or $^{13}\text{C}_3$ -glycochenodeoxycholic acid ($^{13}\text{C}_3$ -GCDCA). Cells have been incubated with liposomes made only with P90G (negative control) and liposomes made with P90G and $^{13}\text{C}_3$ -cholesterol (for 2 and 24 hours in order to select the appropriate incubation time to measure the production of BAs).

The contribution of GCA in the medium to the measured $^{13}\text{C}_3$ -GCA remains constant and the true $^{13}\text{C}_3$ -GCA produced by the cells from $^{13}\text{C}_3$ -cholesterol is clearly measurable above this background after 24 h incubation.