Data supplement: **Characterisation of MDCO-216 drug product**

Fig. I.

Size exclusion chromatography

A 60 µl aliquot of MDCO-216 (Drug Product used for this study) corresponding to 0.84 mg phospholipid and 0.80 mg protein was injected in a Agilent 1100 HPLC chromatographic system equipped with a Superdex 200 HR 10/300 GL column (GE Healthcare/Amersham Biosciences). Mobile phase was 6 mM Sodium phosphate buffer, pH 7.4, 150 mM NaCl. The fractions were collected in Eppendorf tubes prior to processing according to the phospholipids assay instructions.

POPC concentrations in the fractions are plotted in red, protein concentration (as OD 280) plotted in blue. Recovery of phospholipid from the column was 76.2%.
Retention times (peak) for reference proteins on this column are: ferritin 24 min, albumin 31 min.

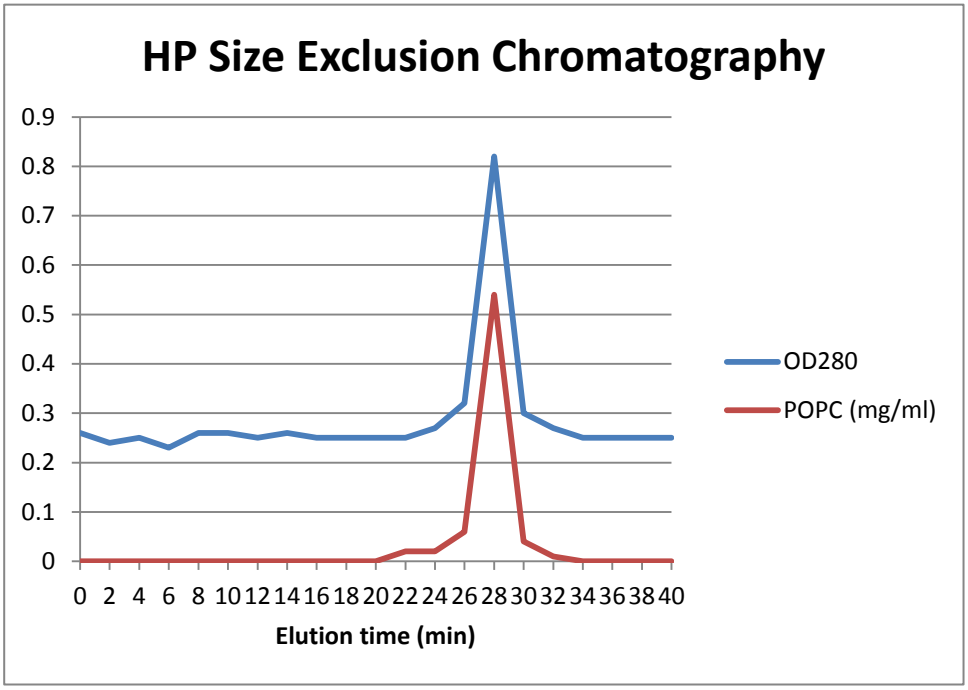
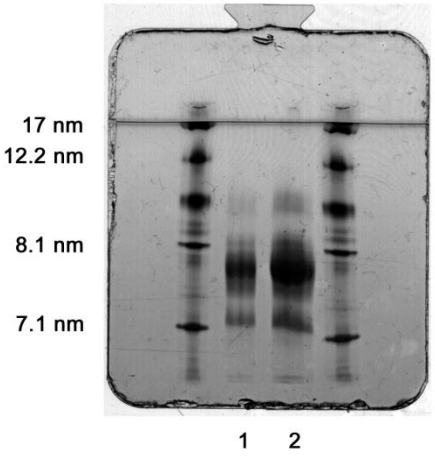


Fig. II.

Non-denaturing polacrylamide gradient gel electrophoresis (PAGGE)

PAGGE was performed using the Phast System as described by Favari et al (46). Staining was done with Coomassie Blue (for protein). Reference proteins (from top): thyroglobulin (17.0 nm), ferritin (12.2 nm), catalase (9.2 nm), lactate dehydrogenase (8.1 nm), and albumin (7.1 nm).



Lanes left and right: Reference proteins

Lane 1: MDCO-216 14 ug (protein)

Lane 2: MDCO-216 28 ug (protein)

To verify the absence of unlipidated protein in the product, MDCO-216 and lipid-free apoA-IMilano were run on a non-denaturing 4-20% polyacrylamide gel, stained with Coomassie Blue R250 and scanned with a GS-690 densitometer (Bio-Rad Laboratories).

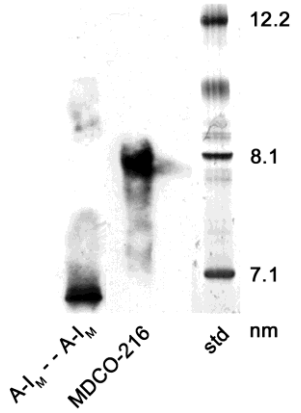


Fig. III

Effect of MDCO-216, mixed with buffer containing BSA on global (J774 cells preincubated with cAMP), basal (J774 cells not pretreated with cAMP), and ABCA1-mediated (difference due to cAMP) cholesterol efflux from J774 cells, and on SR-BI mediated efflux from Fu5AH cells. Final MDCO-216 concentrations during the efflux assays were 14 or 100 µg/ml and final concentration of BSA was 0.5%. Efflux in the presence of BSA only (final BSA concentration 0.5%) was zero in all assays.