J Chem Ed, vol 88, 2011, p. 1580-1 (experimental procedure)

Cholesterol is an important structural component of animal cell membranes. An egg yolk contains about 200 mg of cholesterol, much of it bound by its hydroxyl group to membrane lipids. Methoxide and heat will help free the cholesterol from the lipids so it can be isolated.

In a 250 mL round bottom flask, combine two hard boiled egg yolks, 1 g of K2CO3, 5g of sand, and 10 mL of CH3OH. Using a glass stir rod or a spatula, grind together until it is smooth – it will look like soft scrambled eggs. Add 20 mL of cyclohexane (for azeoptope), stir thoroughly – it will look like corn meal mush – then warm to reflux. Rotovap off the solvent. While the solvent is rotovapping, prepare a chromatography column with 15 g of silica gel and a layer of sand on top, and have 15 test tubes ready to take fractions. Also prepare a mixture of 30 mL of EtOAc and 170 mL of petroleum ether.

To the 250 mL round bottom flask with the evaporated egg mixture, add 30 mL CH2Cl2, and stir thoroughly. Add the Ch2Cl2 solution (not the egg mixture!) to the top of the column, and let the solvent go down on its own. When all of the CH2Cl2 is down, rinse the inside top of the column with a little of the EtOAc/pet ether mixture.

By this time, the solvent should have begun to drip out of the bottom of the column. Add more of the EtOAc/pet ether mixture to the top of the column, and apply gentle pressure to the column as you collect 10 mL fractions. You should collect 15 fractions. Check the fractions by thin layer chromatography. Usually, the cholesterol comes in fractions 6-10. Rotovap the cholesterol fractions in a tarred round bottom flask. You should be rewarded with iridescent rosettes of the product. Record the weight and the melting point of the isolated crude cholesterol.

Prurification of cholesterol: Cholesterol forms a specific 2:1 complex with oxalic acid. Take up your crude cholesterol in 5 mL of 1,2-dichloroethane n a 50 mL Erlenmeyer flask. Add 80 mg of oxalic acid, and heat to reflox. Let the flask cool for 20 minutes and then swirl the flask in ice water. The contents should gel into a mush of crystals. Vacuum filter with 1,2-dicholroethane, suck dry, and spread out to dry. Take up the white residue in a 50 mL Erlenmeyer flask with 5 mL of water. Heat to reflux, chill in ice water, and vacuum filter and vacuum filter. This should give pure white cholesterol, (mp = ??) Record the melting point and weight. Yields are often better if the complexation with oxalic acid is allowed to go overnight.