SEPARATION OF LIPID CLASSES FROM BIOLOGICAL EXTRACTS

Summary: This method separates lipids in up to seven individual classes using Strata NH2 96 well plates

SPE:

Pre-extraction Plate:	Strata C18E 50mg	(P/N 8E-S001-DGB)
Fractionation Plate:	Strata NH2 50mg	(P/N 8E-S009-DGB)
Collection Plate:	Strata Collection	(P/N AH0-7194)
Ultravap 96 well	Ultravap 96	(P/N EH0-7899)

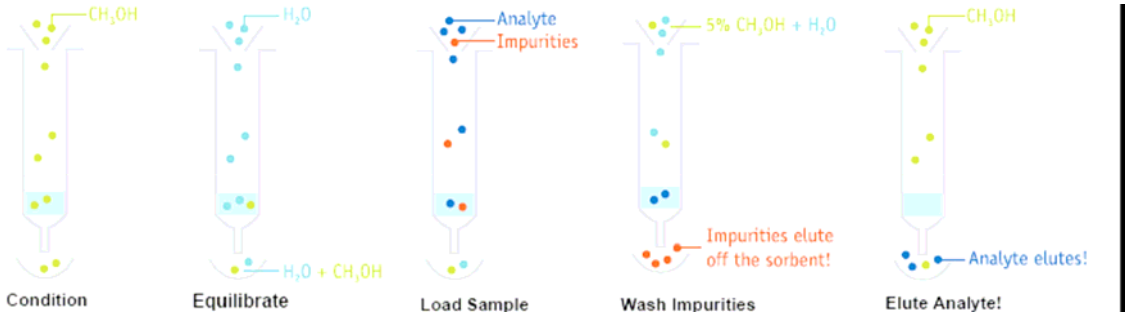
Solvents Needed:

1. Chloroform / 2-propanol (2:1)
2. 2% Acetic acid in diethyl ether
3. 1% Diethyl ether, 10%Methylene chloride in hexane
4. 5% Ethyl acetate in hexane
5. 15%Ethyl acetate in hexane
6. Chloroform / methanol (2:1)
7. Chloroform
8. Hexane
9. Methanol

For preparation of plasma or other biological fluids:

Prepare sample:

Dilute sample 1:1 with PBS solution



Condition	500uL ACN
Equilibrate	500uL water
Load	sample
Wash	500ul 5%MeOH in DI water Dry for 1-2 min

Elute 500uL ACN

Fractionate Lipids

Step 1. Sample Pretreatment: -Evaporate lipids to dryness under nitrogen.
-Dissolve in 50ul. of Chloroform.

Step 2. NH2 Sorbent #1:
CONDITION: Two 200uL portions of Hexane
LOAD:
Vacuum sample through the sorbent.
ELUTE #1 (*neutral lipids*)
-400uL of Chloroform / 2-propanol (2:1). Collect neutral lipids.
-Dry eluate under nitrogen and set aside for further fractionation.
ELUTE #2 (*fatty acids*)
-400uL of 2% Acetic acid in diethyl ether. Collect fatty acid fraction.
ELUTE #3 (*phospholipids*)
-400uL of Methanol. Collect phospholipid fraction.

Step 3. NH2 Sorbent #2:
-Reconstitute dried eluate from Elution #1(neutral lipids) in 0.50uL hexane.
CONDITION: Two 200uL portions of hexane.
LOAD:
Vacuum sample through the sorbent.
ELUTE #4 (*Cholesteryl ester*)
-400uL of hexane. Collect cholesteryl ester fraction.

Step 4. NH2 Sorbent #3:
CONDITION: Two 2mL portions of hexane.
-**Attach NH2 plate #3 <u>below</u> NH2 plate #2**
ELUTE #5 (*triglycerides*)
-600uL of 1% Diethyl ether, 10%Methylene chloride in hexane.
Vacuum through both plates to collect triglyceride fraction.
ELUTE #6 (*Cholesterol*)
-1200uL of 5% Ethyl acetate in hexane. Vacuum through both plates to collect cholesterol fraction.
-Disconnect the stacked plates, keeping only the top plate (NH2 sorbent #2) for further elution.
ELUTE #7 (*diglycerides)*
-400uL of 15%Ethyl acetate in hexane. Collect diglyceride fraction.
ELUTE #8 (*monoglycerides*)
-400uL of Chloroform / methanol (2:1). Collect monoglyceride fraction.