

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)

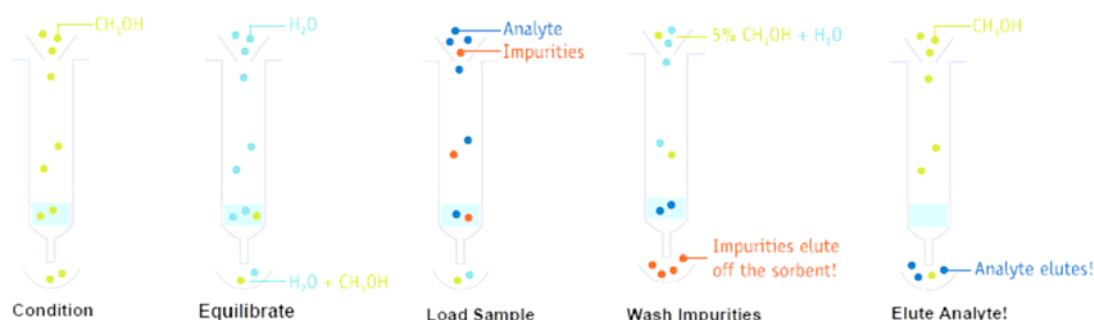
Solvents Needed:

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 below 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.