



# Pholipidec<sup>®</sup>

## Certified Soy Lecithin Column

### Operating Instructions

Soy Lecithin (Phospholipids) HPLC analysis:

#### MATERIALS:

- ❖ HPLC instrumentation:
  - Agilent model 1050 Quaternary Pump
  - Agilent model 1050 Auto-liquid Sampler
  - Agilent model 1050 Solvent Degasser
  - Evaporative Light Scattering Detector, Alltech ELSD-2000, Alltech Associates Inc.
- ❖ Column:
  - Advanced Separation Technologies Inc. (Astec)
  - Pholipidec<sup>®</sup> Certified Soy Lecithin Column (250x4.6mm) with guard column (20x4mm), 5µm silica bonded phase, Catalog No. 59000
- ❖ Reagents:
  - Chloroform, HPLC Grade, Fisher Scientific
  - Methanol, HPLC Grade, Fisher Scientific
  - Water, HPLC Grade, Fisher Scientific
  - Ammonia, ACS Certified, Fisher Scientific
- ❖ Sample Standards (Avanti Polar Lipids, Inc. Alabaster, AL 35007, 1-800-227-0651, E-mail - [info@avantilipids.com](mailto:info@avantilipids.com), [www.avantilipids.com](http://www.avantilipids.com)):
  - Soy Phosphatidylethanolamine (Soy PE)
  - Soy Phosphatidylinositol (Soy PI)
  - Soy Phosphatidylserine (Soy PS)
  - Soy Phosphatidylcholine (Soy PC)
  - Soy Phosphatidic Acid (Soy PA)
  - Soy Lyso Phosphatidylcholine (Soy LPC)

#### PREPARATIONS:

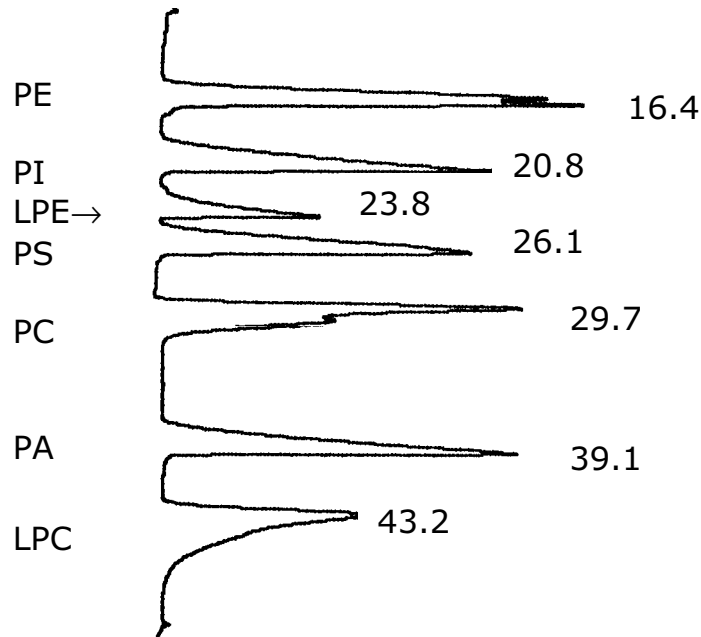
- ❖ Mobile Phases:
  - A: 80/19/1, CHCl<sub>3</sub>/CH<sub>3</sub>OH/NH<sub>4</sub>OH (v/v/v)
  - B: 60/39/1, CHCl<sub>3</sub>/CH<sub>3</sub>OH/NH<sub>4</sub>OH (v/v/v)
  - C: 50/48/1/1, CHCl<sub>3</sub>/CH<sub>3</sub>OH/H<sub>2</sub>O/NH<sub>4</sub>OH (v/v/v/v)
  - D: 100, CH<sub>3</sub>OH
- ❖ Phospholipid Standards:
  - All standards are combined at 2mg/mL each in chloroform.

**PROCEDURE:**

- Detector: Evaporative Light Scattering Detector, Alltech ELSE-2000  
Impactor: ON; Drift Tube Temperature: 45°C; Nitrogen Flow: 1.7 L/min., Gain: 1
- Column conditioning:  
Wash first with 20mL of methanol at 1mL/min.  
Without injection, repeat gradient method 1-2 times before analysis.  
Before storage, wash column and store in methanol.
- Gradient method with flow rate 1.0mL/min:  
Initial: 100% A  
Gradient from 100% A to 100% B over 25 minutes  
Gradient from 100% B to 100% C at 30 minutes  
Hold in 100% C until 45 minutes  
Gradient from 100% C to 100% D at 48 minutes  
Hold in 100% D until 55 minutes  
Gradient to 100% A at 56 minutes  
Hold in 100% A until 65 minutes

**CHROMATOGRAM**

Standard samples with LPE (Lysophosphatidylethanolamine, peak 3)



TO REORDER:

Catalog No.	Description
59000	Pholipidec® LC Column (250 x 4.6mm) with Integral Guard Column
59124	Pholipidec® Replacement Column, 250x4.6mm
59100	Pholipidec® Guard Columns 20.0 x 4.0mm, pack of 4

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