

**Supercritical Fluid Extraction and Chromatography of Various Lipids from
Soybean Lecithin**

By

Shiu-Hang Yip

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science

In

Chemistry

Committee

Larry T. Taylor, Chairman

David G.I. Kingston

Sungsool Wi

August 16, 2007

Blacksburg, Virginia

Key words: supercritical fluid chromatography, supercritical fluid extraction,
phospholipids, ethylpyridine phase

ABSTRACT

Phospholipids are commonly found in biological membranes. They have a polar head group and two ester linked fatty acids tails. Different methods such as thin layer chromatography and high performance liquid chromatography coupled with ultraviolet, refractive index, flame ionization detector, and mass spectrometry (MS) detection have long been used in the study of phospholipids. These methods were time-consuming and lacked quantitative accuracy. In this work, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine have been studied by supercritical fluid chromatography (SFC) coupled with evaporative light scattering detection (ELSD) and mass spectrometry (MS). Four different silica-based stationary phases were studied: 2-ethylpyridine, 4-ethylpyridine, diol and conventional cyanopropyl. The influence of different mobile phase additives on the elution of phospholipids has been studied. The results have shown that isopropylamine is a better additive compared with ammonium acetate, tetrabutyl-ammonium acetate, and trifluoroacetic acid for the elution of phospholipids. All phospholipids have been eluted with baseline separation in less than 15 minutes although there is some partial overlap on the pyridine columns. The second goal for this work was fractionation of phospholipids from lecithin (a by-product from soybean) by using supercritical fluid extraction (SFE) with methanol-modified CO₂. Neutral lipids were first removed from the crude sample using pure CO₂. Partial fractionation of PE and nearly pure fractionated PC were obtained by varying the modifier concentration in the extraction fluid at 460 atm and 40°C with silica gel inside the extraction vessel. A total of six components were isolated from crude soybean lecithin.