Separation of Lipids by Normal Phase Flash Chromatography

Chromatography Application Note AN05

Application Overview

Lipid mixtures can be difficult to separate and frequently require multiple procedures to achieve a high purity. With an Isco Normal Phase Redi*Sep*® column and Combi*Flash*® chromatography system, this once difficult procedure can be performed with ease.

For this application, a mixture was prepared containing non-polar lipids, glycolipids, and phospholipids.

With Combi*Flash* gradient former, it is possible to allow less polar species to selectively elute first and more polar species to elute later. When a gradient system is used in normal phase chromatography, the "A" solvent is the less polar solvent, gradually the more polar solvent "B" is mixed in, this allows the species to be separated by their respective polarities. In this case, hexane is used for solvent A and ethyl acetate for solvent B.

General Method

An Isco Combi*Flash* Sq16x was used according to routine procedures. Prior to separation, a Redi*Sep* 120g disposable flash column was equilibrated with 400 mL of 100% solvent A, hexane. The top of the column was then disconnected from the instrument and a 1 mL portion of the fluid sample was taken directly from the lipid vial. This 1 mL portion was directly injected onto the top of the column.

This method of column loading is used when the sample to be purified contains undissolved bulk matter that is unimportant to the separation. The bulk matter will stay on top of the column while the relevant components flow through and are separated.

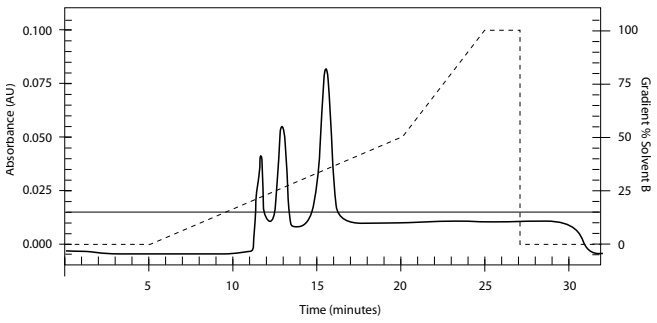

Figure 1: Lipid Chromatography

Analytical Results

Refer to Table 1 and Figure 1. The lipid mixture was separated and collected in three separate high purity fractions, unattended, in less than 30 minutes.

Summary

With the Combi*Flash* Sq 16x and Normal Phase 120g Redi*Sep* disposable flash column, a complex separation can be easily carried out. A distinct difference in the polarity between lipid components allows separation to occur with a gradient system. This method benefits from the use of gradient and fully automated equipment.

Roughly estimating by peak area, it appears that the first two peaks each make up 25% of the total peak area and the third peak makes up about 50%.

The separation took less than twenty minutes. The capability to save, optimize and repeat a specialized method can be a significant saver of time, solvent, and materials.

Table 1: Lipid Separation

Wavelength	24 nm	
Solvent A:	Hexane	
Solvent B:	Ethyl acetate	
Gradient:	% Solvent B	Minutes
	0	Initial
	0	5.0
	50	15.0
	100	5.0
	100	2.0
	05	0.0
	0	5.0
Run time:	32.0 minutes	

Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
www.teledyneisco.com