Fast and Efficient Purification of a Mixture of Glycerides, Mono-, Di-, and Tristearin

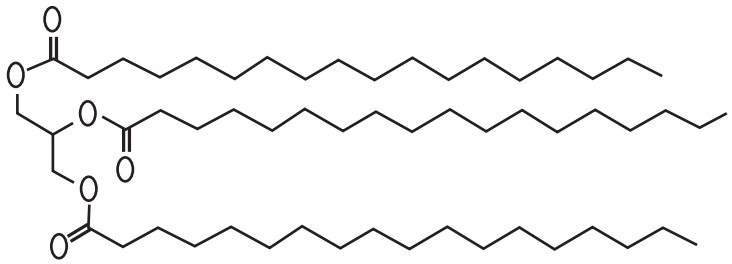
Reveleris® X2 Flash Chromatography System

Introduction

Triglycerides are fatty acids present as esters in combination with glycerol.[1] Tristearin, a glyceryl ester of stearic acid, is a simple triglyceride having three identical acyl chains. It is found in both plants and animals and may be used as a drug delivery vehicle for target drug compounds.

This application demonstrates purification of a mixture of glycerides (mono-, di-, and tristearin) using C18 and amino phase chemistries. The C18 phase uses hydrophobic interaction to determine separation, wheras the amino phase uses lipophilic interaction between the stationary phase and the fatty acyl chain of the analyte to determine separation. The amino phase cartridge can be used in a normal phase or a reversed phase mode, depending on the solvents employed. When used in normal phase mode, the less polar compounds elute first, followed by the elution of the polar ones selectively.

Tristearin



Experimental

Run Conditions

Cartridge: Reveleris® C18 12g (PN: 5152103)	**Cartridge:** Reveleris® Amino 12g (PN: 5157331)
Load: 0.4% mass load on column	**Load:** 0.4% mass load on column
Flow rate: 30 mL/min	**Flow rate:** 30 mL/min
Equilibration: 5.0 min	**Equilibration:** 5.0 min
Solvent A: Acetonitrile	**Solvent A:** Acetonitrile
Solvent B: Methylene Chloride	**Solvent B:** Methylene Chloride
Detection:	**Detection:**
UV 1: 210 nm	UV 1: 210 nm
UV 2: 254 nm	UV 2: 254 nm
ELSD	ELSD
Run time: 11 minutes	**Run time:** 13 minutes

Gradient Method		
Step	**Time (min.)**	**%B**
1	0	10
2	8	100
3	3	100

Gradient Method		
Step	**Time (min.)**	**%B**
1	0	100
2	2	100
3	9	10
4	2	10

References

[1] *Dewick, Paul., Medicinal Natural Products: A Biosynthetic Approach, 3rd edition, (2009), John Wiley and Sons, UK, pp 40-44.*

Results and Discussion

Using the Reveleris® C18 cartridge and a methylene chloride/acetonitrile solvent gradient, all three components were separated in less than ten minutes (fig. 1). Due to the baseline drift from the methylene chloride and the non-chromophoric nature of the compounds, the UV detector failed to detect and collect the peak fractions. The evaporative light scattering detector (ELSD), an integral part of RevealX™ detection technology, detected all three peaks and triggered fraction collection during the separation. Purification of this mixture with UV-only detection would require collecting all the fractions based on time or volume, followed by subsequent thin layer chromatography (TLC) confirmation of each fraction. This would take significantly longer and may result in sample loss.

Using the same solvent combination with the Reveleris® amino cartridge, the selectivity of these compounds was altered by using a stronger nonpolar solvent and then gradually increasing the polarity of the mobile phase with acetonitrile. This allowed the tristearin to elute earlier, while the polar components were retained longer (fig. 2). Such a change in selectivity may enable recovery of the most important compounds from a mixture in a shorter time with high purity.

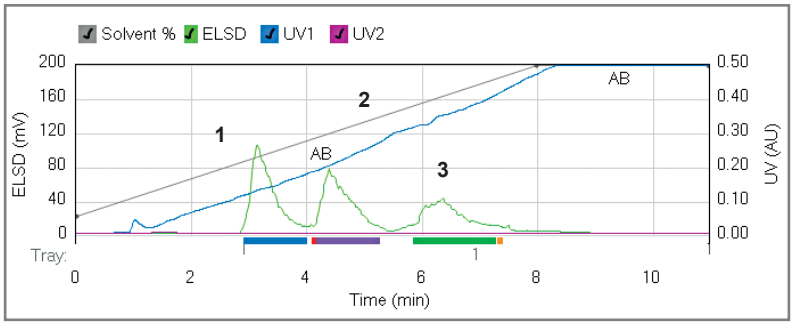


Figure 1: Separation of mono-, di-, and triglycerides using a Reveleris® C18 cartridge.

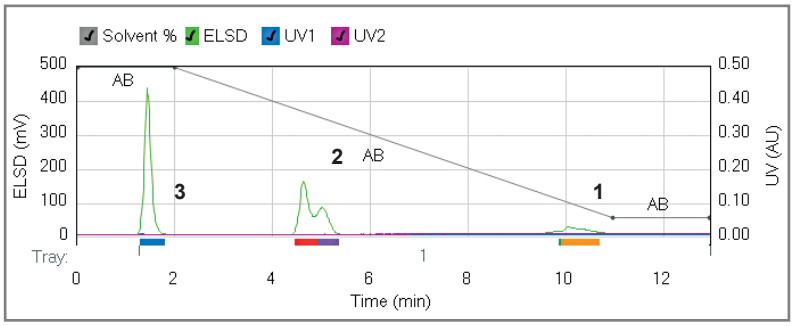


Figure 2: Separation of mono-, di-, and triglycerides using a Reveleris® amino cartridge. Note how altering polarity affects elution order.

Compound ID:
1. Monostearin
2. Distearin
3. Tristearin

Conclusion

Using the Reveleris® X2 Flash Chromatography System with selective Reveleris® cartridge phases, method development can be optimized for efficiency and timesavings. Modifying gradients to further affect elution or retention of compounds provides additional options to separate challenging compounds. The RevealX™ detection technology in the Reveleris® X2 Flash Chromatography System helps chemists to isolate and purify lipid-based compounds that are non-chromophoric with speed and high purity.

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