Fast and Efficient Purification of Fatty Acid Methyl Esters (FAMEs)

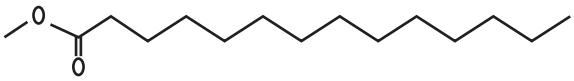
Reveleris® X2 Flash Chromatography System

Introduction

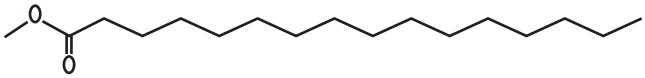
Lipids play a major role in biological functions due to their presence in all cells.[1] They are categorized into various classes and subclasses of molecules based on their hydrophobic and hydrophilic elements. Fatty acids are commonly found in natural product extracts and have been shown to interfere with noncellular assays. Fatty acid esters are fatty molecules containing nonpolar groups and may be used in drug development for lipid-based formulations.

Purifying complex mixtures containing non-chromophoric compounds such as lipids can be difficult. Traditional ultraviolet (UV) detection fails to detect lipid targets and impurities that are present at low levels or lack chromophores, requiring a 'collect all' approach that can add significant time to the process. With RevealX™ detection technology in the Reveleris® X2 flash system, chemists can purify lipid-based compounds that are non-chromophoric with speed and high purity.

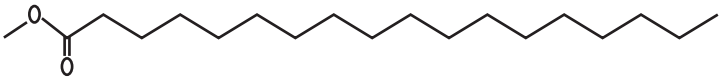
1) Methyl Myristate



2) Methyl Palmitate



3) Methyl Stearate



Experimental

Run Conditions

Cartridge: Reveleris® C18 12g (PN: 5152103)
Load: 1.1% mass load on column
Flow rate: 30 mL/min
Equilibration: 5.0 min
Solvent A: Methanol
Solvent B: Acetonitrile
Detection:
UV 1: 200 nm
UV 2: 220 nm
ELSD
Run time: 8 minutes

Gradient Method

Step	Time (min.)	%B
1	0	90
2	8	90

References

[1] *Eoin Fahy et al.; 2005. A comprehensive classification for lipids. J. Lipid Res. 46: 839-861.*

Results and Discussion

This application demonstrates purification of a mixture of fatty acid methyl esters with the Reveleris® X2 Flash Chromatography System. Using the C18 bonded reversed phase Reveleris® cartridge and an acetonitrile/methanol solvent system, the peaks were baseline resolved and easily detected by the ELSD (evaporative light scattering detector) (fig. 1). Using the ELSD, a key component of RevealX™ detection technology, the three compounds collected as three pure fractions may be further dried for subsequent testing or chemical synthesis. Without RevealX™ detection technology, purification of the three compounds would not have been possible in a single step. 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) testing of each fraction. This would take significantly longer and may result in sample loss.

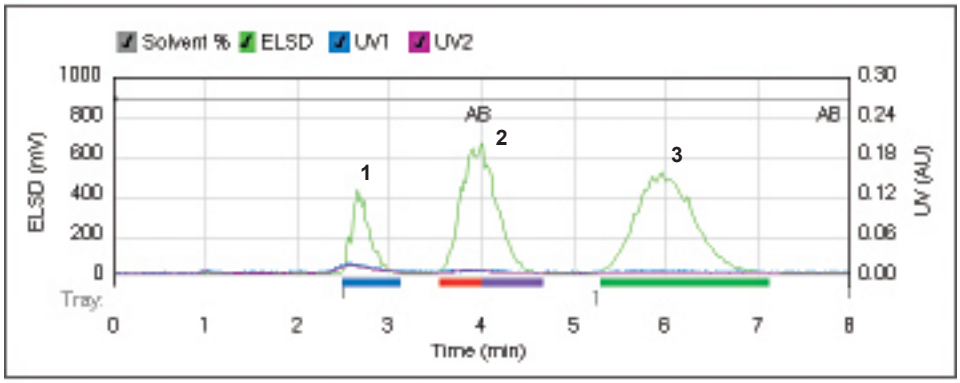


Figure 1: The methyl esters of the three fatty acids are separated using isocratic condition in less than eight minutes. All three compounds lack a UV chromophore but are fully detected by ELSD.

Compound ID:
1. Methyl Myristate
2. Methyl Palmitate
3. Methyl Stearate

Conclusion

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. Reveleris® reversed phase cartridges allow baseline resolution of the three lipid components, helping to maximize purity. The separation is fast and efficient, resulting in three pure fractions within 8 minutes, meaning more time can be spent on discovery and less on purification.

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