Fast and Efficient Purification of Sterols

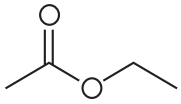
Reveleris® X2 Flash Chromatography System

Introduction

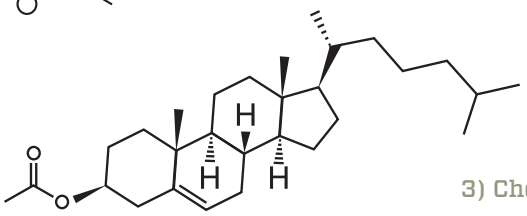
Sterols are waxy insoluble substances or lipids synthesized from acetyl coenzyme A (CoA).[1] The most common sterol found among humans is cholesterol; though there are other non-cholesterol sterols derived from plants (phytosterol). Cholesteryl ester is the major transport and storage form of cholesterol in lipoprotein particles and most cells. Research work in sterols is of high interest to better understand human diseases such as atherosclerosis and in development of novel drugs to treat coronary heart disease.

Separation and identification of cholesterol and cholesterol-based compounds is challenging. They lack chromophores and have poor response due to higher background interference from certain organic solvents when detected at low wavelengths. Purification of such compounds may require additional method development with other solvents or a 'collect all' approach, adding time to the purification process. This application shows the benefit of RevealX™ detection technology for purifying a mixture of non-chromophoric sterols.

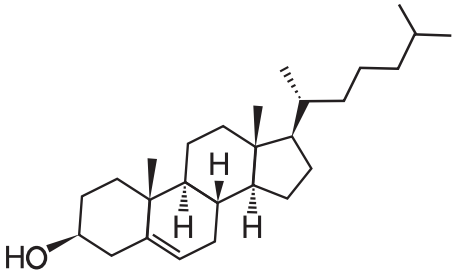
1) Ethyl Acetate



2) Cholesteryl Acetate



3) Cholesterol



Experimental

Run Conditions

Cartridge: Reveleris® C18 12g (PN: 5152103)
Load: 2.2% mass load on column
Flow rate: 30 mL/min
Equilibration: 5.0 min
Solvent A: Acetonitrile
Solvent B: Methylene Chloride
Detection:
UV 1: 210 nm
UV 2: 254 nm
ELSD
Run time: 8 minutes

Gradient Method

Step	Time (min.)	%B
1	0	30
2	8	100

References

[1] *Davidson, M. H.; Toth, P. P.; Maki, K. C.; Therapeutic Lipidology: Phytosterolemia; Humana Press, Totowa, New Jersey, 2007, pp 291-319.*

Results and Discussion

As sterols are hydrophobic and not easily soluble in polar solvents, the use of non-polar solvents such as methylene chloride is often required. When monitoring a purification by traditional UV detection at low wavelengths, the baseline signal drifts, giving poor sensitivity for fraction collection and completely masking peak number 3 (fig. 1). This results in extra time needed for post run work-up of all of the fractions.

Using RevealX™ detection technology, both UV and evaporative light scattering detection (ELSD) can be used to detect peaks and trigger fraction collection. In this run, ethyl acetate is detected by UV, while the cholesteryl acetate and cholesterol peaks are detected by ELSD (fig. 2). As there is no baseline drift in the ELSD signal, peaks can be collected based on the signal triggered from the ELSD while monitoring them with UV. This allows for separation and collection of all three components in a single run with high purity in less than six minutes.

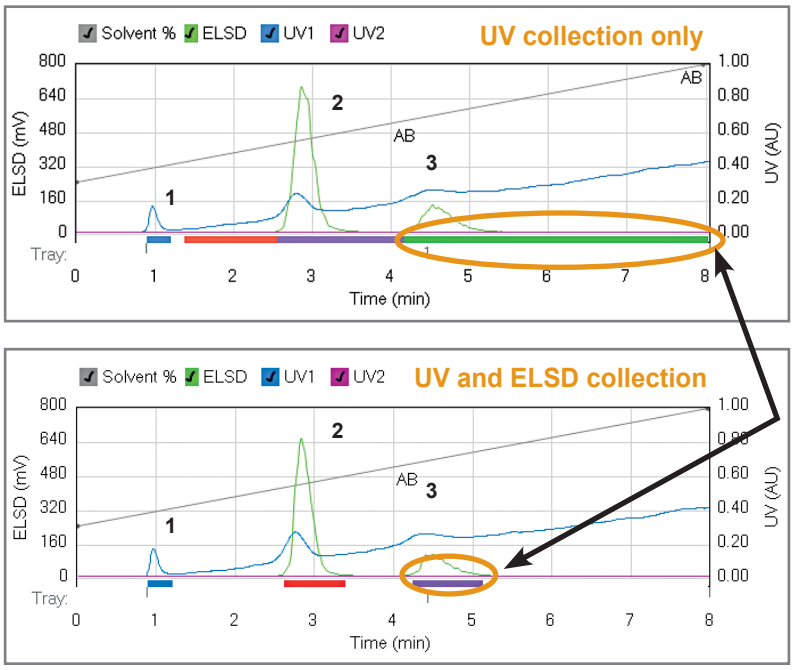


Figure 1: Fractions are collected continuously when using a UV detector only. Baseline drift masks peak 3.

Figure 2: With RevealX™ detection technology, the peaks can be monitored by UV detector while fractions are collected based on the ELSD signal. With ELSD, peak 3 is fully baseline resolved.

The integrated detection of RevealX™ detection technology gives confirmation of results by both UV and ELSD in a single run.

Compound ID:
1. Ethyl Acetate
2. Cholesteryl Acetate
3. Cholesterol

Conclusion

The RevealX™ detection technology in the Reveleris® X2 Flash Chromatography System allows chemists to isolate and purify sterol-based compounds that are non-chromophoric with speed and high purity.

The separation is fast and efficient, resulting in three pure fractions within six minutes, meaning more time can be spent on discovery and less on purification.

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