

Bile Acid Purification During Lead Generation in Drug Discovery

Reveleris® iES Flash Chromatography System

Introduction

Bile acids, such as cholic acid, are steroidal acids secreted into the gastrointestinal tract to emulsify fats and encourage digestion.¹ Hepatic conversion of cholesterol into bile acids increases the breakdown of LDL cholesterol, adding value in treatment of coronary patients. Bile acids are starting materials for the semi-synthesis of other medicinal steroids due to their availability as raw materials. Conjugation of cholic acid with glycine or taurine increases

its water solubility for drug development.

Using the RevealX™ technology of the Reveleris® iES flash chromatography system and the Reveleris® reversed-phase cartridge, chromophoric and non-chromophoric target compounds can be separated and isolated in the presence of small impurities present in a chemical reaction by a single chromatography run.

Experimental

Run Conditions

Cartridge: Reveleris® C18

Solvent A: Water

Solvent B: Methanol

Flow rate: 36 mL/min (12g), 40 mL/min (40g)

UV1 wavelength: 254 nm

UV2 wavelength: 220 nm

ELSD carrier: Iso-propanol

Equilibration: 3.0 min

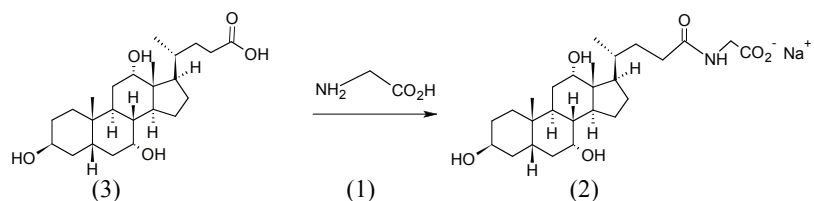
Injection type: Liquid



Results and Discussion

1) Separation of sodium glycocholate from cholic acid and glycine on a 12g Reveleris® C18 flash cartridge

Gradient Method		
Step	Time (min.)	%B
1	0	30
2	7	100
3	1	100



1. Glycine 2mg
2. Sodium Glycocholate 20mg
3. Cholic Acid 2mg

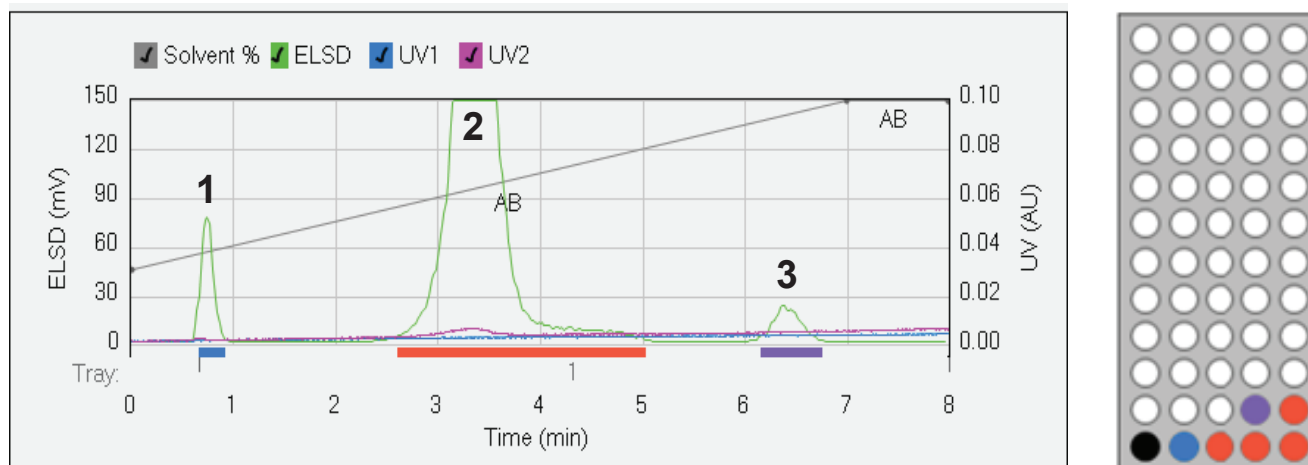


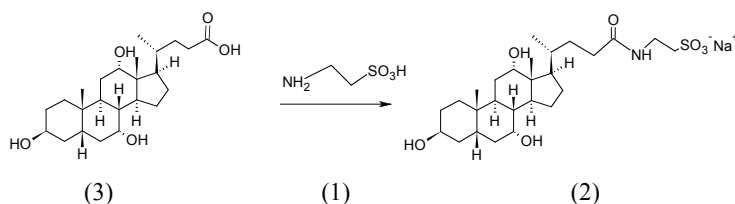
Figure 1: Using traditional flash chromatography system, non-chromophoric compounds have poor sensitivity or are undetected during purification. RevealX™ technology of the Reveleris® iES system can isolate such compounds using the ELSD in a reaction mixture during purification.



Results and Discussion

2) Separation of sodium taurocholate from cholic acid and taurine on a 40g Reveleris® C18 flash cartridge

Gradient Method		
Step	Time (min.)	%B
1	0	30
2	7	100
3	3	100



1. Taurine 1mg
2. Sodium Taurocholate 400mg
3. Cholic Acid 30mg

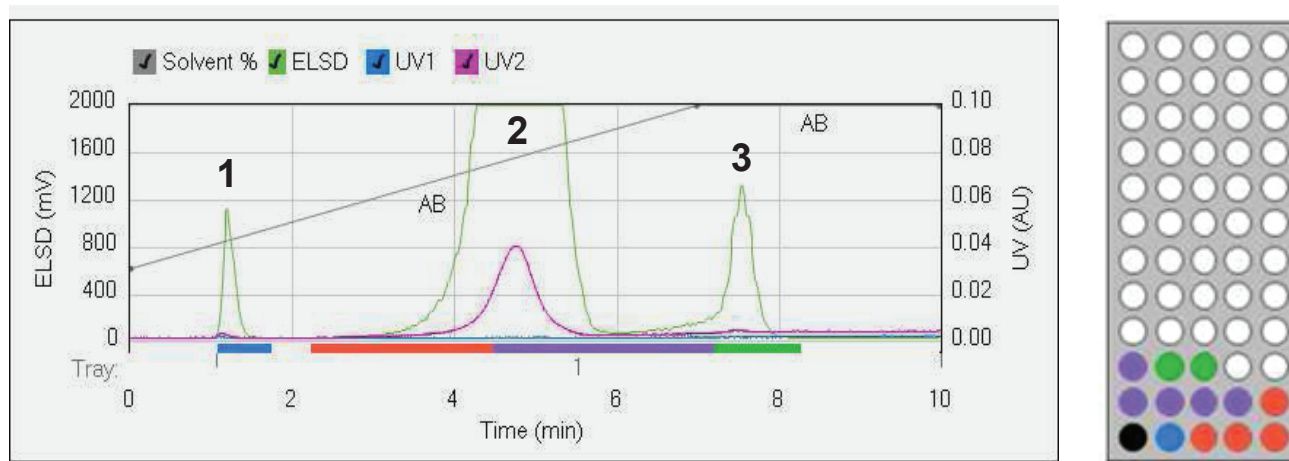


Figure 2: Non-chromophoric compounds on a larger scale are purified using the higher capacity Reveleris® cartridge and the evaporative light scattering detection of the RevealX™ technology.

Conclusion

Using the high sensitivity Reveleris® iES flash chromatography system, all 3 components in a sample mixture have been separated and purified in a single run. Isolation of amounts as low as 1mg and as high as 400mg have been detected here using the evaporative

light scattering detector along with the UV detectors of the RevealX™ technology. Such isolation of compounds from their impurities provides accurate purity and quantity assessment, reducing post-run analysis time.

References

1. Medicinal natural products; a biosynthetic approach, 3rd edition; Dewick, P.; John Wiley & Sons, Inc., Hoboken, New Jersey, 2009.

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