

Reveleris® X2 Flash System

Bile Acid Purification During Lead Generation in Drug Discovery

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.

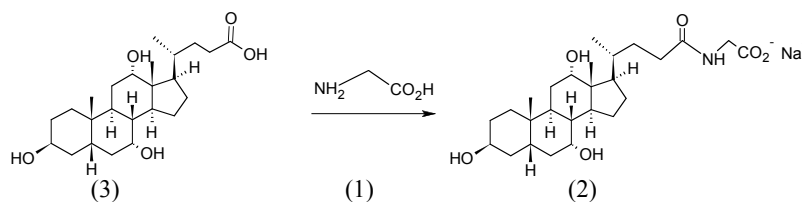
Using the RevealX™ technology of the Reveleris® X2 flash chromatography system and the Reveleris® reversed-phase cartridge, chromophoric and non-chromophoric target compounds can be separated and isolated in the presence of small impurities present in a chemical reaction by a single chromatography run.

Flash Chromatography Conditions:

Cartridge: Reveleris® C18
 Solvent A: Water
 Solvent B: Methanol
 Flow Rate: 36mL/min (12g),
 40mL/min (40g)
 UV1 Wavelength: 254nm
 UV2 Wavelength: 220nm
 Cartridge Equilibration: 3.0 min
 Injection Type: Liquid

Results

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



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

Gradient Table

Step	Time (min.)	%B
1	0.0	30
2	7.0	100
3	1.0	100

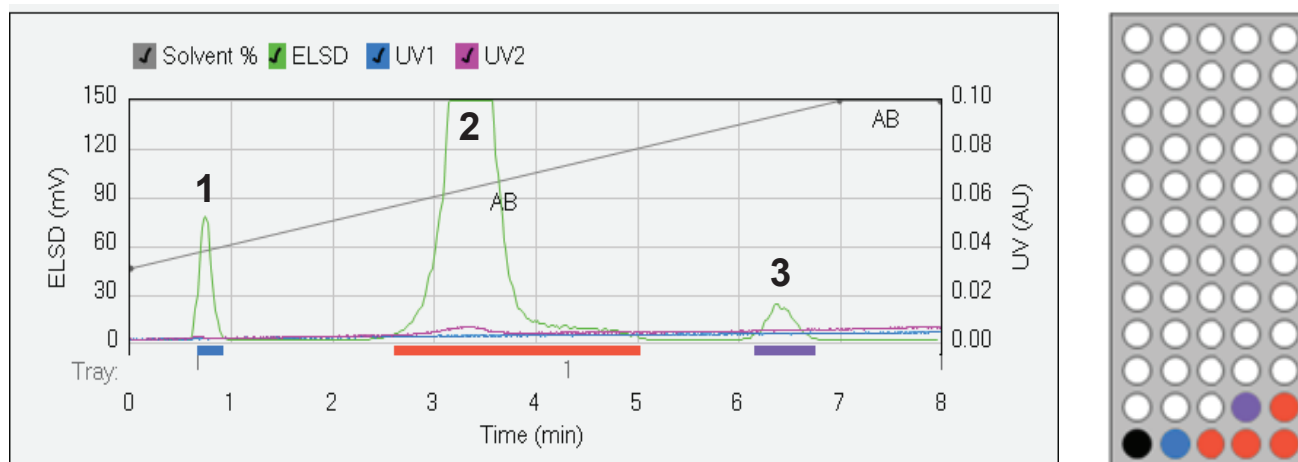
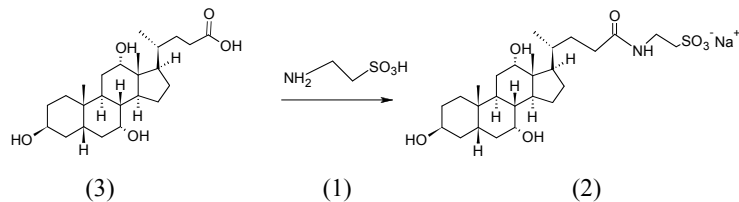


Figure 1: Using traditional flash chromatography system, non-chromophoric compounds have poor sensitivity or are undetected during purification. RevealX™ technology of the Reveleris® X2 system can isolate such compounds using the ELSD in a reaction mixture during purification.

Results continued

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



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

Gradient Table		
Step	Time (min.)	%B
1	0.0	30
2	7.0	100
3	3.0	100

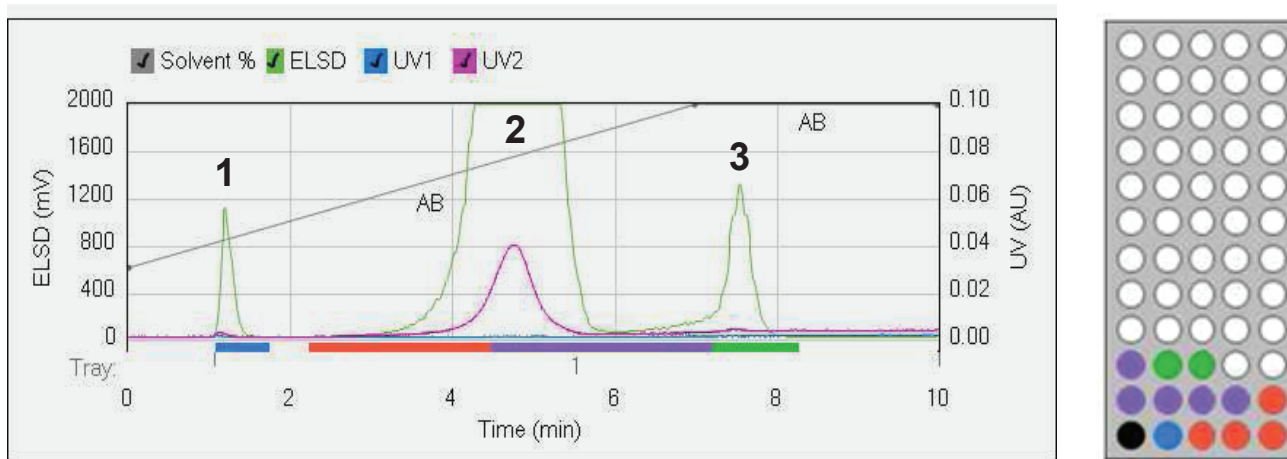


Figure 2: Non-chromophoric compounds on a larger scale are purified using the higher capacity Reveleris® cartridge and the evaporative light scattering detection of the RevealX™ technology.

Conclusion

Using the high sensitivity Reveleris® X2 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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11/2013 M346a

W. R. Grace & Co.-Conn. 7500 Grace Drive, Columbia, MD 21044

Grace Discovery Sciences Regional Headquarters:

IN THE AMERICAS:
2051 Waukegan Road
Deerfield, IL 60015-1899
Tel: 1 847 948 8600
Fax: 1 847.948.1078
Email: discoverysciences@grace.com

IN EUROPE:
Brandstraat 12
B-9160 Lokeren, Belgium
Tel: +32 (0)9-340-65 65
Fax: +32 (0)9 340 65 60
Email: discoverysciences.EU@grace.com

IN ASIA:
19th Floor, K.Wah Center
1010 Huai Hai Zhong Road
Shanghai, 200031 PRC
Tel: 86 21 5467 4678
Fax: 86 21 5405 1500
Email: dsbiz.asia@grace.com

IN INDIA:
17 Commerce Center
Opposite Krishna Hospital, Paud Road
Pune, 411038 India
Tel: +91 20 6644 9900
Fax: +91 20 2544 1740
Email: pune@grace.com

IN AUSTRALIA/NEW ZEALAND:
2 Kerr Court
Rowville, 3178
Victoria, Australia
Tel: +61 3 9237 6100
Fax: +61 3 9237 6101
Email: discoverysciences.AU@grace.com

www.discoverysciences.com

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