Mestranol Purification During Chemical Synthesis Using Reveleris® Navigator™ Software

Reveleris® X2 Flash Chromatography System

Introduction

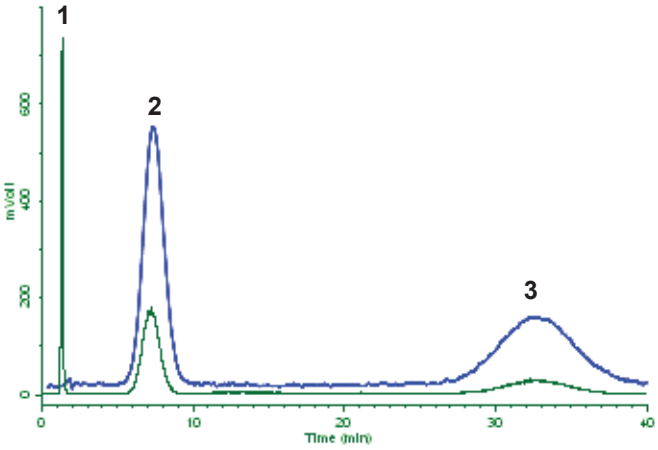
Oestrogen compounds, along with progestogens, are the basis of combined oral contraceptives and hormone replacement therapy (HRT).[1] Among women, they are used to supplement natural oestrogen levels, suppress androgen formation in tumor growth of cancers, along with protection against osteoporosis, heart attacks, and possibly Alzheimer's disease.

Mestranol is one such compound which acts as a pro-drug that is formed from ethynylestradiol and methylsulfate. In this separation, mestranol can be purified using a simple method optimized by the Reveleris® Navigator™ software from the other two components that may be present in the chemical reaction as impurities.[2]

Experimental

Step 1

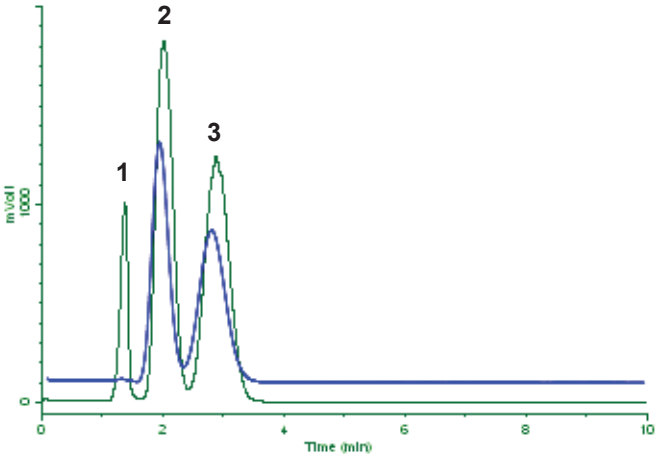
Initially two separate isocratic runs at different methanol concentrations were carried out on an HPLC system using a Reveleris® C18 analytical column. The retention times for the peaks to be resolved were obtained from the run. The chromatography conditions were as listed below.



Run Conditions

Cartridge: Reveleris® C18, 150 x 4.6 mm
Mobile phase: A: Water 20% **B:** Methanol 80%
Flow rate: 1.0 mL/min
Detector 1: UV @ 254 nm
Detector 2: ELSD (55° C, 1.5 LPM, Gain 1)

1. Methyl Sulfate (RT: 1.46 min, 1.26 min)
2. 17α - Ethynylestradiol (RT: 7.04 min, 1.88 min)
3. Mestranol



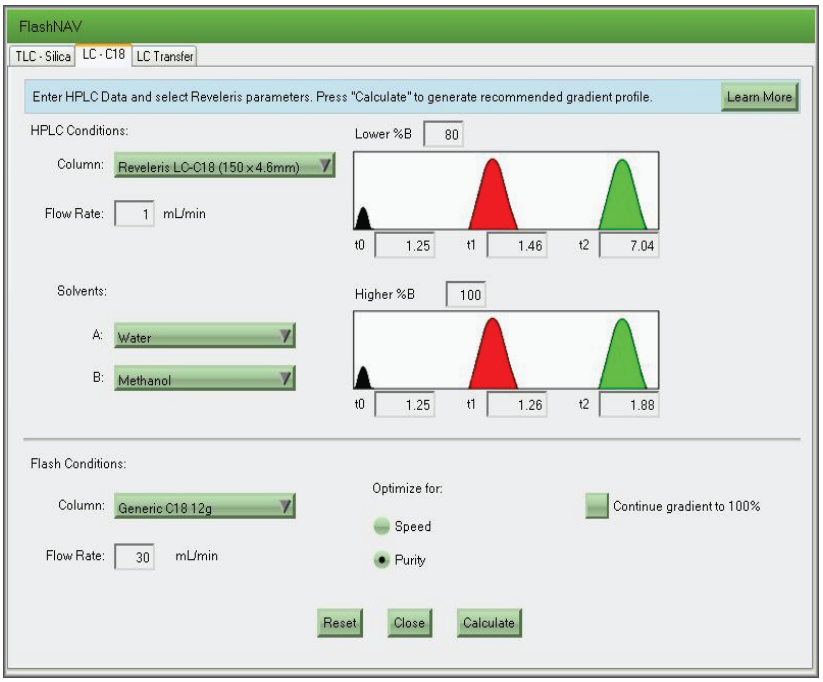
Run Conditions

Cartridge: Reveleris® C18, 150 x 4.6 mm
Mobile phase: A: Water 0% **B:** Methanol 100%
Flow rate: 1.0 mL/min
Detector 1: UV @ 254 nm
Detector 2: ELSD (55° C, 1.5 LPM, Gain 1)

Experimental

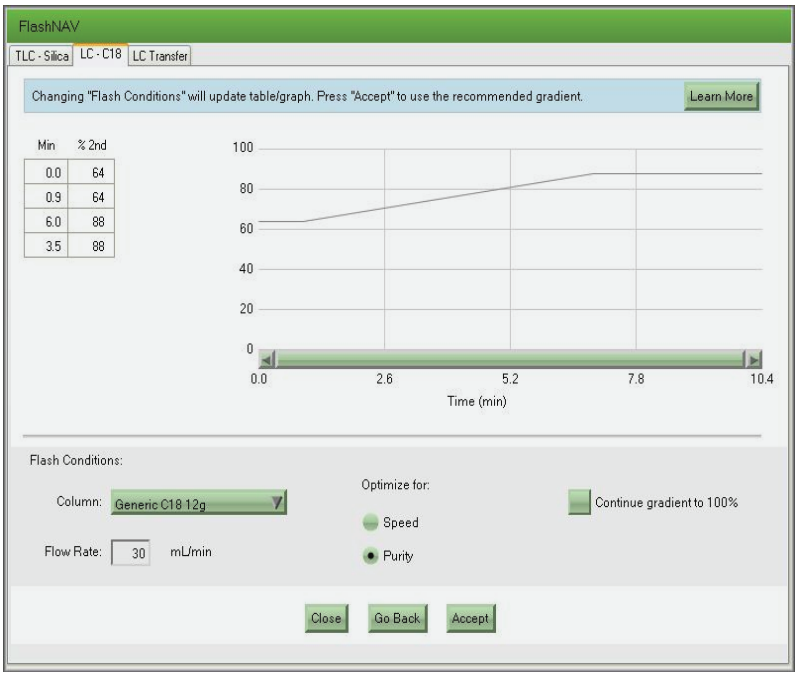
Step 2

After selecting the appropriate HPLC conditions, the values for methanol solvent as percent B mobile phase concentration and the retention times of the peak of interest were entered. The preferred flash column and optimize for purity were selected. Only the retention times of the first two eluted peaks were used for flash chromatography optimization.



Step 3

The Reveleris® Navigator™ software calculated the recommended gradient method to optimize for purity.



Results and Discussion

Run Conditions

Cartridge: Reveleris® C18 12g
Solvent A: Water
Solvent B: Methanol
Flow rate: 30 mL/min
UV1 wavelength: 220 nm
UV2 wavelength: 280 nm
Equilibration: 3.0 min
Injection type: Liquid

Gradient Method		
Step	**Time (min.)**	**%B**
1	0	64
2	0.9	64
3	6	88
4	3.5	88

1. Methyl Sulfate 2.5mg
2. 17α - Ethynylestradiol 2.5mg
3. Mestranol 2.5mg

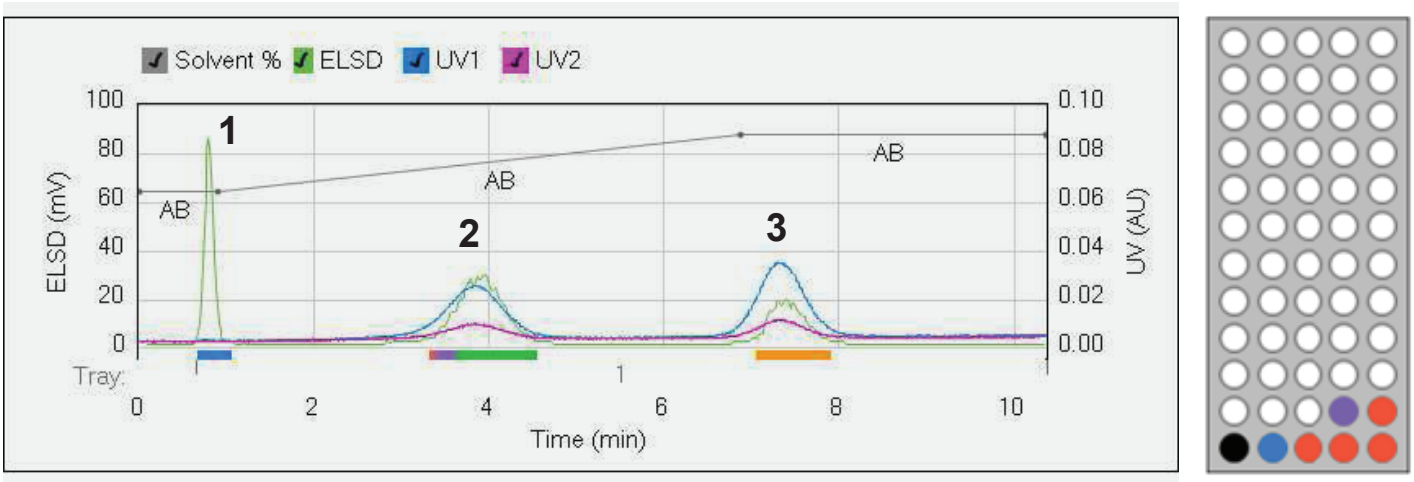


Figure 1: Using a traditional flash chromatography system, chromatography method optimization is dependent on the user. Reveleris® Navigator™ software optimized the method purification by offering the appropriate gradient method for high purity isolation of all the three components that were present in the sample mixture.

Results and Discussion

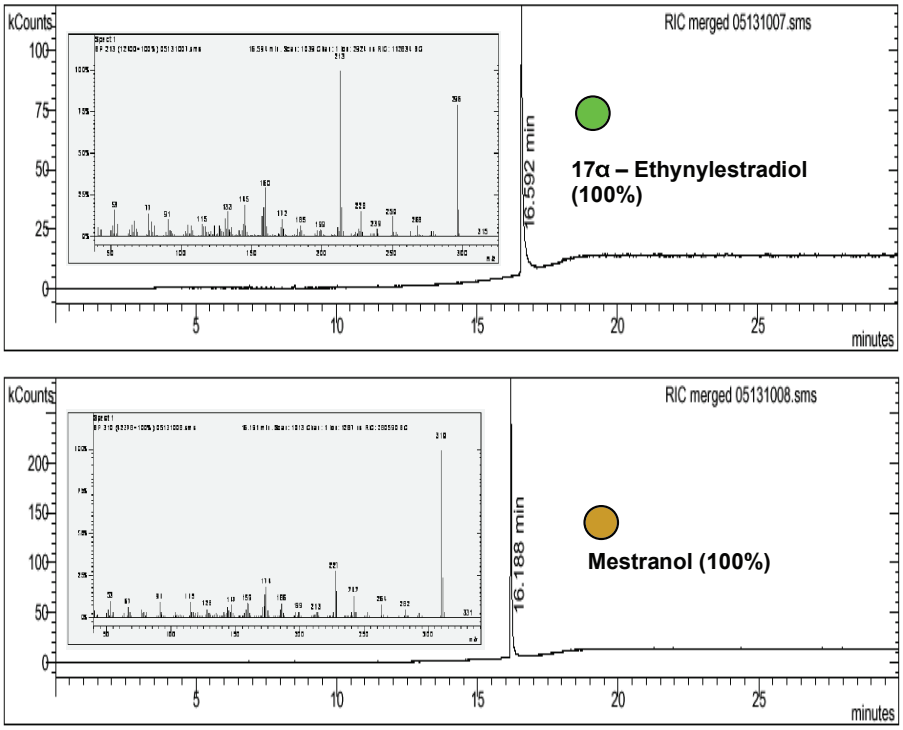


Figure 2: Analysis of mestranol and ethynylestradiol fractions show 100% purity using gas chromatography-mass spectrometry (GC-MS).

GC-MS Conditions

AT-5ms (30 m, 0.25 mm, 0.25 µm)

2 µl injection at 250° C

30:1 split, 0.8 mL/min He

Conclusion

Using the Reveleris® Navigator™ software of the Reveleris® X2 flash chromatography system, mestranol in a sample mixture has been well resolved with high purity from the other two components without any flash method development. Such an optimization tool minimized any sample loss and eliminated any additional chromatographic runs using the flash system.

References

1. Medicinal natural products; a biosynthetic approach, 3rd edition; Dewick, P.; John Wiley & Sons, Inc., Hoboken, New Jersey, 2009.
2. Lesniowski, A.; McCreary, D.; Anderson, S.; Lawrence, K.; Automating gradient method development in flash chromatography provides productivity gains and minimizes solvent usage; ACS Fall 2010 poster, Boston, MA.

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