Improved Purification of Lead Generation Compounds by Flash Chromatography

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Abstract

During pharmaceutical development of biologically active compounds, the identification and purification of unknown impurities is a key requirement for the successful registration of a new molecular entity. Traditional flash chromatography, as preferred by synthetic chemists, is equipped with Ultraviolet (UV) detection that fails to detect targets and impurities that are either present at low levels or lack chromophores. This may result in producing impure targets and can lead to false hits during biotesting.

This study investigates the detection and quantification of an impurity in the presence of the lead compound during purification using multiple signal-processing from UV and ELSD (Evaporative Light Scattering Detector). Using the RevealX™ detection technology in the Reveleris® flash chromatography system, chemists can detect both chromophoric and non-chromophoric compounds present in the sample matrix. A system comparison to a preparative liquid chromatography with an ELSD shows that Reveleris® can be more productive during purification for lead generation.

Background

Identification of small-molecules and transforming them into high-content lead series is a major activity in modern drug discovery.[1] Success in lead optimization leading towards clinical trails depends on the right decisions made in the early stages of the drug discovery process. High numbers of clinical failures have shifted the focus towards flexible, fast, and cost effective strategies to control the rate of attrition.

As new hit and lead generation processes emerge to keep up with the demand in high throughput screening on vast libraries of new targets, the quality of a lead candidate dictates the success during the drug development process. Chemical integrity and overall characteristics of compounds are necessary to evaluate the usefulness of it for a hit-to-lead (HTL) program making it a good candidate for a medicinal chemist to proceed in the laboratory.

Some of the objectives in the lead generation process include the validation of structure and purity of the hit and the use of chromatographic techniques for purity assessment.[2] Problems due to decomposed samples and incorrect structure in the libraries can lead to failures to achieve effective dosage and potency during clinical trials. This has led to the use of more productive and effective purification techniques such as the Reveleris® iES flash chromatography system during the drug discovery process.

Purpose

This study investigates the detection and quantification of an impurity in the presence of the lead compound during purification using multiple signal-processing from UV and ELSD (Evaporative Light Scattering Detector).

Using the RevealX™ detection technology in the Reveleris® flash chromatography system, chemists can detect both chromophoric and non-chromophoric compounds present in the sample matrix.

A system comparison to a preparative liquid chromatography with an ELSD shows that the Reveleris® flash chromatography system can be more productive during purification for lead generation. Advanced method development software which easily optimizes speed and purity of purification methods will be highlighted.

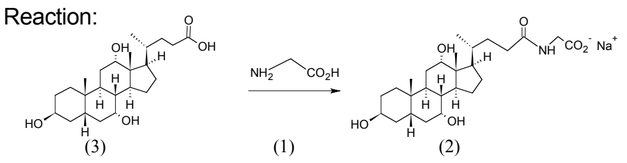
Experimental and Results

Bile Acid Purification During Lead Generation in Drug Discovery

Bile acids such as cholic acid are steroidal acids which occur in salt form in bile, secreted into the gut to emulsify fats and encourage digestion.[3] Conjugation with glycine increases its water solubility. Metabolism to bile acids is the principal way to degrade cholesterol absorbed from the diet.

For cardiovascular compounds, higher sensitivity detection for lead generation compounds using the Reveleris® iES flash chromatography system purifies sodium glycocholate from glycine and cholic acid using a 12g Reveleris® C18 cartridge.

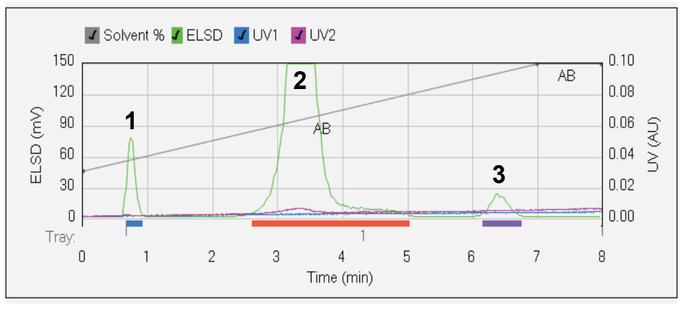
Reaction:



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

Cartridge: Reveleris® C18 media 12g
Flow Rate: 36mL/min
Equilibration: 3.0 min
Run Length: 8.0 min
UV1 Wavelength: 254nm
UV2 Wavelength: 220nm
ELSD Carrier: Iso-propanol
Solvent A: Water
Solvent B: Methanol



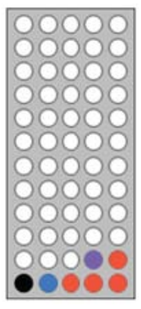


Figure 1: Using traditional flash chromatography system, non-chromophoric compounds have poor sensitivity or are undetected during purification. The RevealX™ technology of the Reveleris® iES system can isolate such compounds using the ELSD in a reaction mixture during purification.

Using the Reveleris® iES flash chromatography system, all 3 components can be separated and purified in a single run for further synthesis towards a lead product during drug discovery process. Amounts as low as 2mg can be detected and collected with the RevealX™ technology.

Bile Acid Purification continued

Hepatic conversion of cholesterol into bile acids increases the breakdown of LDL cholesterol, adding value in treatment of coronary patients.[3] 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 taurine increases its water solubility.

Purification of sodium taurocholate from taurine and cholic acid has been done using a 40g Reveleris® C18 cartridge and the Reveleris® iES flash system.

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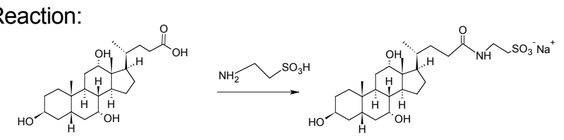


(3) (1) (2)

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

Cartridge: Reveleris® C18 media 40g
Flow Rate: 40mL/min
Equilibration: 5.0 min
Run Length: 10.0 min
UV1 Wavelength: 254nm
UV2 Wavelength: 220nm
ELSD Carrier: Iso-propanol
Solvent A: Water
Solvent B: Methanol

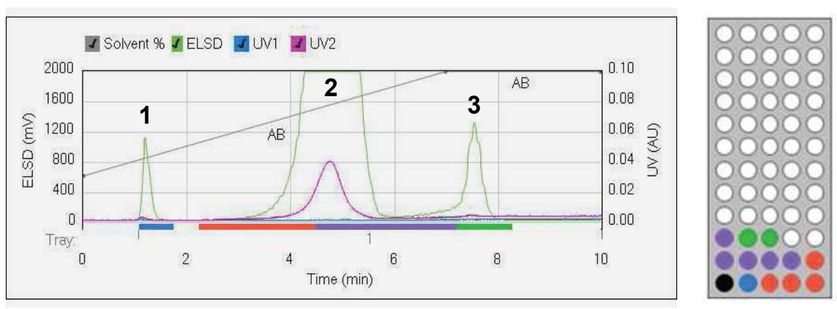


Figure 2: Non-chromophoric compounds on a larger scale are purified using the higher capacity Reveleris® cartridge and the evaporative light scattering detection of the RevealX™ technology.

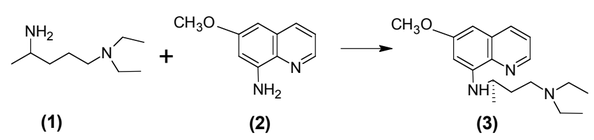
The Reveleris® iES system can purify target drug compounds in the presence of small impurities from a chemical reaction in a single run by using a 40 gram cartridge size and the RevealX™ technology.

RevealX™ Technology in Anti-malarial Drug Purification in Drug Discovery

Cinchona and its alkaloids, such as quinine, have been used for the treatment of malaria, a disease caused by protozoa.[3] A range of synthetic anti-malarial drugs has been produced as alternatives to quinine. The emergence of Plasmodium falciparum strains resistant to the synthetic drugs has resulted in reintroduction of quinine, such as the Primaquine with 8-aminoquinoline, in drug discovery.

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 small amounts present in a chemical reaction by a single chromatography run.

Reaction:



(1) **(2)** **(3)**

1. 2 Amino-5-Diethylamino Pentane 20mg
2. 8-Amino-6-MethoxyQuinoline HBr 1mg
3. Primaquine 10mg

Gradient Table		
Step	**Time (min.)**	**%B**
1	0.0	30
2	5.0	100
3	7.0	100

Cartridge: Reveleris® C18 media 12g
Flow Rate: 36mL/min
Equilibration: 5.0 min
Run Length: 12.0 min
UV1 Wavelength: 254nm
UV2 Wavelength: 220nm
ELSD Carrier: Iso-propanol
Solvent A: Water
Solvent B: Methanol

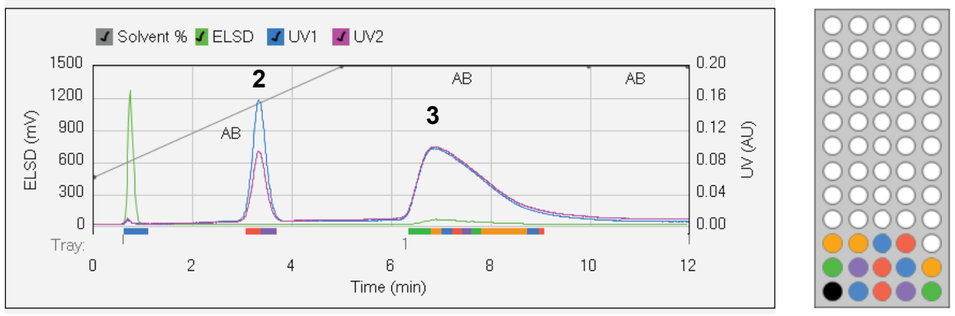


Figure 3: Using traditional flash chromatography system, non-chromophoric compounds have poor sensitivity or are undetected during purification. The RevealX™ technology of the Reveleris® iES system can isolate such compounds using the ELSD in a reaction mixture during purification.

Using the RevealX™ technology, separation of both chromophoric and non-chromophoric compounds can improve sample recovery and reduce post-run analysis time in the drug discovery and development process.

RevealX™ Technology in Impurity Isolation During Drug Purification

Impurity isolation is critical during drug discovery.[2] The purity of samples is compromised at times to meet the demands of increasing high throughput screening. This can produce high false positive hit rates, potentially wasting time and resources. Due to the stringent requirements from the ICH, all impurities need to be identified and reported, even at low levels.

Using the RevealX™ technology of the Reveleris® iES flash chromatography system, chromophoric and non-chromophoric target compounds are isolated using multiple detection technology and advanced signal processing to recognize peaks and collect compounds during chromatography.

Gradient Table		
Step	**Time (min.)**	**%B**
1	0.0	20
2	7.0	100
3	5.0	100

Cartridge: Reveleris® C18 media 12g
Solvent A: Water
Solvent B: Methanol
Flow Rate: 36mL/min
UV1 Wavelength: 254nm
UV2 Wavelength: 220nm
ELSD Carrier: Iso-propanol
Cartridge Equilibration: 3.0 min
Injection Type: Liquid

1.16-β Hydroxy boldenone
2.Impurity

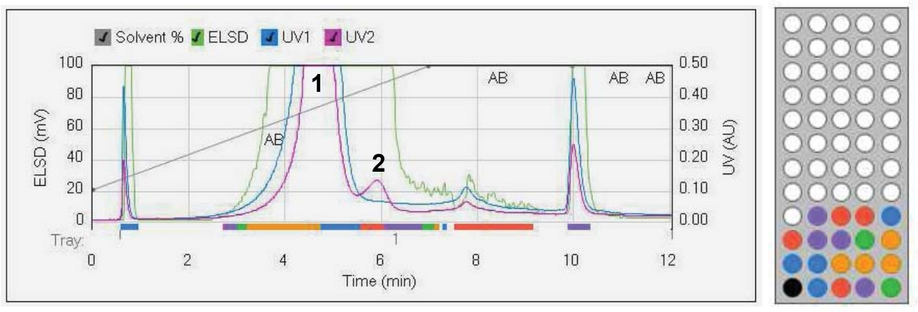


Figure 4: The Reveleris® iES system shows the co-elution of an impurity peak as detected by the RevealX™ technology. Due to the high ELSD response for 16-β Hydroxy boldenone, the non-chromophoric impurity component is detected only by the secondary wavelength of the UV detector at 220nm.

Using the ELSD along with the dual UV detectors of the RevealX™ technology, isolation of impurities at low levels is possible during a chromatographic run.

Mestranol Purification During Chemical Synthesis Using Reveleris® Navigator Software

Oestrogen compounds, along with progestogens, are the basis of combined oral contraceptives and hormone replacement therapy (HRT).[3] Among women, they are used to supplement natural oestrogen levels, suppress androgen formation in tumor growth of cancers, and are also used to help provide 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.[4]

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.

HPLC Conditions:
Column: Reveleris® C18 media, 150 x 4.6mm
Mobile Phase A: Water 20% **B:** Methanol 80%
Flow Rate: 1.0mL/min
Detector 1: UV @ 254nm
Detector 2: ELSD (55º C, 1.5LPM, Gain 1)

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

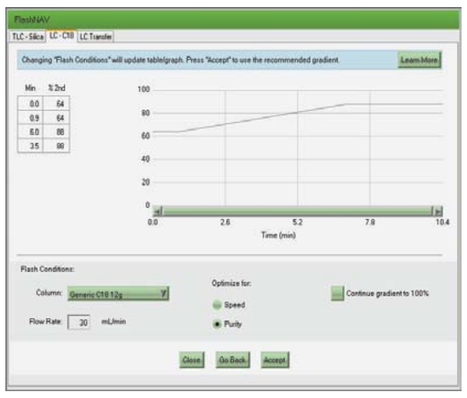
HPLC Conditions:
Column: Reveleris® C18 media, 150 x 4.6mm
Mobile Phase A: Water 0% **B:** Methanol 100%
Flow Rate: 1.0mL/min
Detector 1: UV @ 254nm
Detector 2: ELSD (55º C, 1.5LPM, Gain 1)

Mestranol Purification continued

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.

Experimental - Step 3:



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

Cartridge: Reveleris® C18 media 12g
Solvent A: Water
Solvent B: Methanol
Flow Rate: 30mL/min
UV1 Wavelength: 220nm
UV2 Wavelength: 280nm
ELSD Carrier: Iso-propanol
Cartridge Equilibration: 3.0 min
Injection Type: Liquid

Gradient Table		
Step	**Time (min.)**	**%B**
1	0.0	64
2	0.9	64
3	6.0	88
4	3.5	88

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

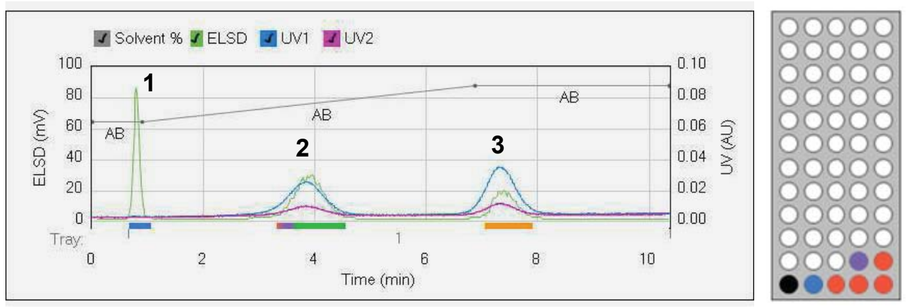
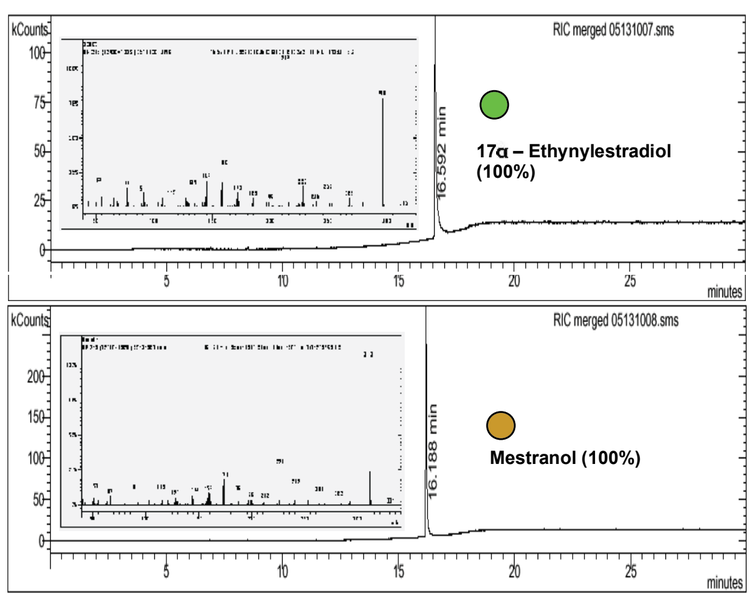


Figure 5: Using a traditional flash chromatography system, chromatography method optimization is dependent on the user. Reveleris® Navigator software optimized the method purification by offering the appropriate gradient method for high purity isolation of all the three components that were present in the sample mixture.



GC-MS Conditions:
AT-5ms (30m, 0.25mm, 0.25µm)
2µl injection at 250°C
30:1 split, 0.8mL/min He

Figure 6: Analysis of mestranol and ethynylestradiol fractions show 100% purity using gas chromatography-mass spectrometry (GC-MS).

Using the Navigator software of the Reveleris® iES 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 sample loss and eliminated additional chromatographic runs using the flash system.

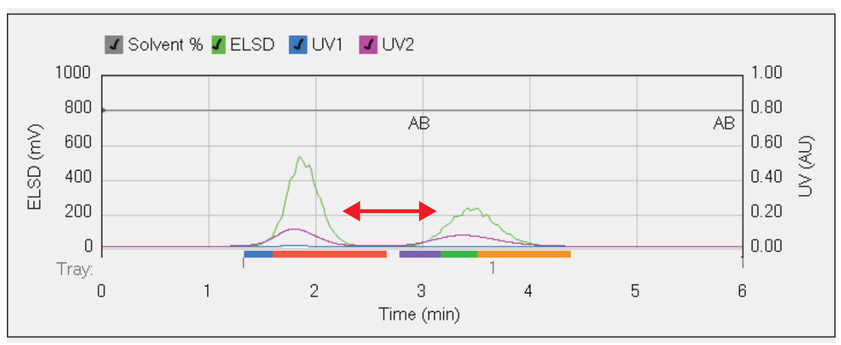
Flash Chromatography Can be More Productive Than Preparative Chromatography During Drug Purification

The Reveleris® iES flash chromatography system detects and collects smaller quantities of compounds for scientists whose applications demand increased lab productivity during purification in the drug discovery process.

Purification bottlenecks encountered when using flash chromatography can be eliminated by using the RevealX™ detection technology of the Reveleris® iES system, which independently triggers fraction collection from multiple detectors including UV and ELSD. Equipped with high performance flash cartridges and universal RevealX™ detection, chemists can isolate target compounds and low-level impurities from their chemical mixture, saving time and labor.

Using the Reveleris® Navigator optimizer tool helps minimize guesswork for the user during method development for flash chromatography, helping to save both time and sample. Using a Reveleris® iES system offers multiple benefits, such as having an integrated fraction collector, user-friendly software, the Reveleris® Navigator optimizer tool, and higher capacity cartridges, making it simple and more productive compared to a preparative chromatography system.

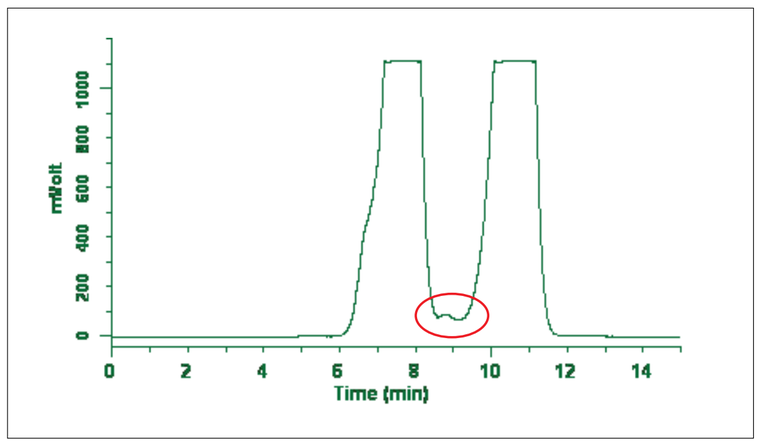
Flash Chromatography



Flash Cartridge: Reveleris® C18 media 40g
Solvent A: Water
Solvent B: Methanol
Flow Rate: 40mL/min
UV1 Wavelength: 254nm
UV2 Wavelength: 220nm

ELSD Carrier: Iso-propanol
Cartridge Equilibration: 5.6 min
Injection Type: Liquid
Mobile Phase: 20:80 Water:Methanol
Sample: 1mL injection of 10mg/mL Estriol and Estradiol each

Preparative Chromatography



Preparative Column: 250 x 22mm
Mobile Phase: 20:80 Water:Methanol
Detector: ELSD (Gas: 1.5L/min, Drift Tube: 40ºC, Gain: 1)

	Recovery in mg	
	Flash	Preparative
Estriol	7	5
Estradiol	5	2

Peaks lose baseline resolution on a preparative column compared to flash chromatography separation.

Due to lower capacity of the preparative column, multiple injections are required to purify larger quantity of samples. Injected peaks begin to co-elute on the standard reversed-phase preparative column, losing baseline resolution. With higher capacity flash cartridges, samples with solubility issues can be purified in a single run using flash chromatography. Flash chromatography allows higher loading using a Reveleris® cartridge over the preparative column. At 100% purity, recovery of the two compounds was higher on the Reveleris® system than on the preparative system. Integrated RevealX™ technology with fraction collector made it less tedious and time consuming for collecting ELSD fractions.

Benefits of Reveleris Include:

- **RevealX™ technology**
- **Navigator optimizer**
- **Integrated fraction collector**
- **Higher loading**
- **Higher purity**
- **Disposable columns**
- **User friendly instrument**
- **Time saver**

Conclusion

The Reveleris® iES flash chromatography system is designed to detect and collect smaller quantities of compounds for scientists whose applications demand increased lab productivity during purification in the drug discovery process.

Equipped with high performance flash cartridges and universal RevealX™ detection, chemists can isolate target compounds and low level impurities from their chemical mixture, helping to save time and labor. Purification bottlenecks encountered when using flash chromatography can be eliminated by using the RevealX™ detection technology of the Reveleris® iES system, which independently triggers fraction collection from multiple detectors including UV and ELSD.

Using the Navigator optimizer tool helps to eliminate guesswork for the user during method development for flash chromatography, helping to save time and sample. Isolating larger quantities of the target compound can therefore become simple and productive when compared to a preparative chromatography system.

References

1. Bleicher K. H.; Böhm H.; Müller K.; Alanine, A. I.; Hit and Lead generation: Beyond high-throughout screening, Nature Review, Vol. 2, May 2003, pp. 369-378.
2. Lead generation approaches in drug discovery; Rankovich, Z. and Morphy, R.; John Wiley & Sons, Inc. Hoboken, New Jersey, 2010.
3. Medicinal natural products; a biosynthetic approach, 3rd edition; Dewick, P.; John Wiley & Sons, Inc., Hoboken, New Jersey, 2009.
4. 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.