Reveleris® X2 Navigator™ Software Improves Purification Efficiency and Productivity

Introduction

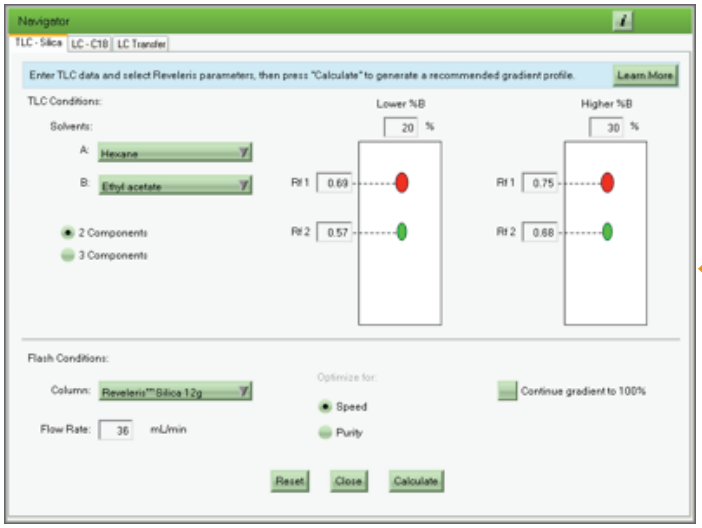
The Reveleris® X2 Navigator™ software module is a unique and powerful software tool that enables chemists to optimize purification of their target compounds by delivering fast, high quality separations at minimal cost.

Flexible and intuitive, the Reveleris® X2 Navigator™ software is essential for multi-user and environmentally conscience labs seeking first-time purification success, increased throughput, and lower operating costs.

Users can quickly build high quality methods that help prevent sample loss and rework, detrimental to any research program. Use of the Reveleris® X2 Navigator™ software puts the focus on discovery and not on chromatography.

To access the Reveleris® X2 Navigator™ software, press the Reveleris® X2 Navigator™ software button located above the gradient display. The Reveleris® X2 Navigator™ method options are selected and the TLC Rf inputs are entered in the appropriate fields for both the target and neighboring impurities.

Accessing the Reveleris® X2 Navigator™ Software



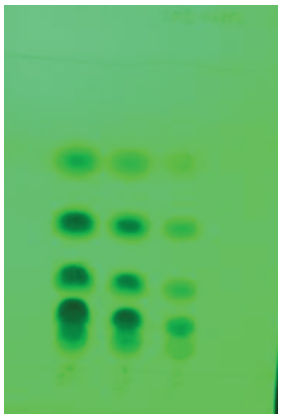
Reveleris® X2 Navigator™ TLC Input Screen

Experimental

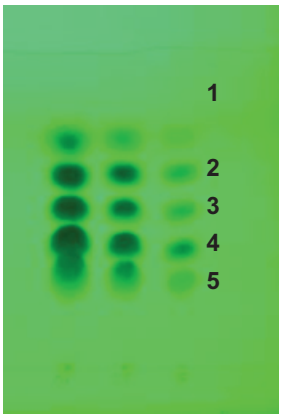
A 5-component test mixture was prepared to evaluate the performance of the Reveleris® X2 Navigator™ software based on
TLC Rf value input. The standards (**Table 1**) were purchased from Sigma Aldrich, St. Louis, MO.

Five grams of each component was dissolved in 50mL of acetone and then 50mL of hexane was added to give a total concentration of 50mg/mL for each component. 50g of dry silica was added to the solution and the solvent was evaporated resulting in the crude sample adsorbed onto the silica surface (approximately 75g total mass). Drying crude sample solutions onto silica allows low solubility samples to be loaded onto flash columns without affecting the chromatography with strong dissolution solvents, typical of liquid loading. In this application 1g of pre-adsorbed silica (corresponding to 333mg actual sample mixture) was loaded onto the cartridges.

TLC analysis was performed using two different ratios of hexane/ethyl acetate (80:20 and 60:40). Resulting Rf values were used as inputs in the Reveleris® X2 Navigator™ software to predict the gradient conditions. The TLC plates, visualized under UV 254nm, are shown below:



80:20 Hexane:Ethyl Acetate



60:40 Hexane:Ethyl Acetate

Figure 1: Test mixture sample analysis by thin layer chromatography

Component	g	mg/mL	Rf 80:20	Rf 60:40
Napthalene	5	50	0.66	0.72
1-nitronapthalene	5	50	0.46	0.60
2-nitroaniline	5	50	0.26	0.48
3-nitroaniline	5	50	0.16	0.38
4-nitroaniline	5	50	0.10	0.27

Table 1: 5-component test mix

Three different Reveleris® X2 Navigator™ methods were evaluated to determine how the options of optimizing on speed and/or purity,
and number of closely eluting spots (choice of 1 or 2), affect the success of the purification using the 5-component test mixture:

Method A: Optimized using SPEED; 2 COMPONENTS - spot 3 (target compound) and spot 4 (impurity)

Method B: Optimized using PURITY; 3 COMPONENTS - spot 3 (target compound) and spots 2 & 4 (impurities)

Method C: Optimized using PURITY; 2 COMPONENTS - spot 3 (target compound) and spot 2 (impurity)

Results and Discussion

In flash purification, it is important that target compounds and any contaminants are detected and well separated. Relying on traditional optimization techniques comes with the risks of failed fraction triggering, excessive run times, and reduced recoveries.

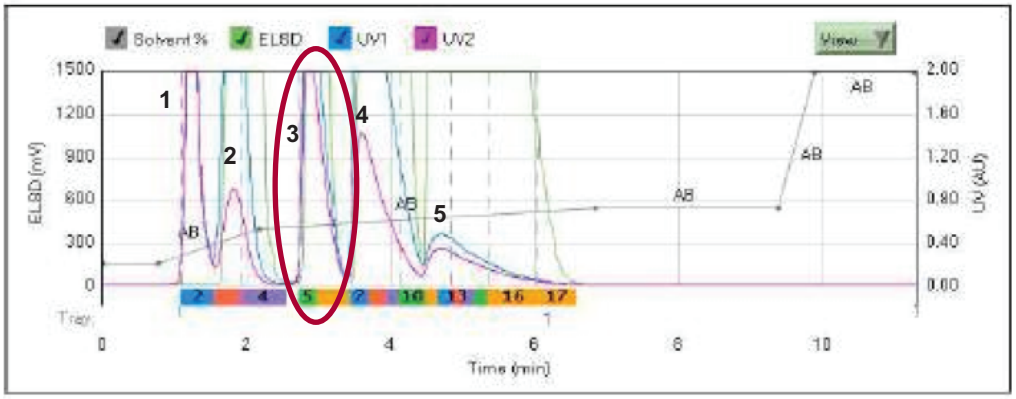
The Reveleris® X2 Navigator™ software significantly improves flash separations by delivering both high purity and maximum quantity of promising target compounds by allowing selective optimization on speed or purity. Simple inputs from two TLC plate analyses are all that is needed for the patent-pending algorithm of the Reveleris® X2 Navigator™ software to predict purification run success.

Method A

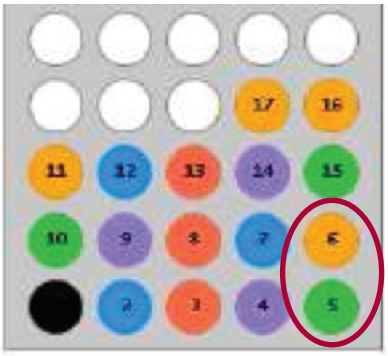
Typically in the SPEED option, as shown in Method A, the chemist may be isolating an intermediate and therefore may not be as concerned about maximizing purity or yield. The SPEED option allows chemists to quickly isolate and access their target intermediate required for the next synthetic conversion. The SPEED option can also help reduce solvent consumption.

Method A: Optimized using SPEED; 2 COMPONENTS - spot 3 (target compound) and spot 4 (impurity)

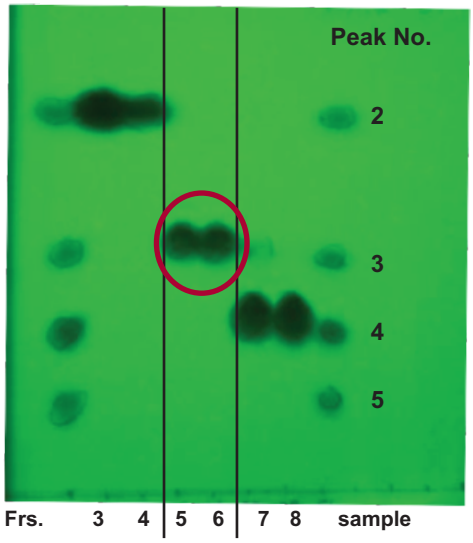
Gradient Method			
Step	**Min**	**Solvents**	**%B**
1	0.0	AB	10
2	0.8	AB	10
3	1.4	AB	26
4	4.7	AB	36
5	2.6	AB	36
6	0.5	AB	100
7	1.4	AB	100



Flash Purification of 5-component Crude mixture using Method A



Method A collected fractions



Method A Fraction Analysis by TLC

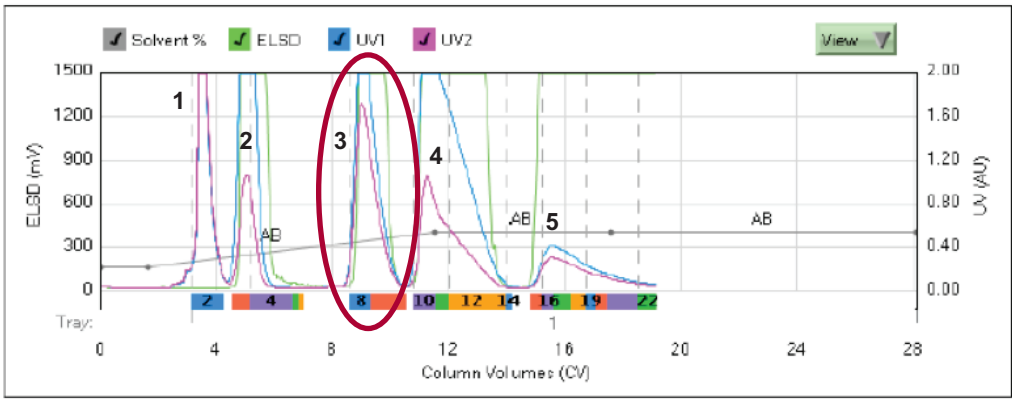
In Method A, peak 3 (target) is adequately resolved from neighboring peaks (impurities) in about 6 minutes. Excellent separation is observed between peak 2 and peak 3. Peak 3 was collected in fractions 5 and 6 and appears to be pure by TLC analysis. A small amount of peak 3 remains in fraction 7 due to incomplete baseline separation.

Method B

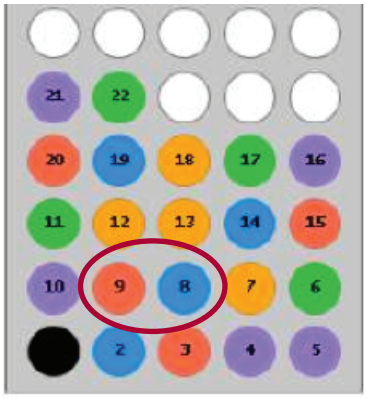
If purity and yield are most important, then the PURITY method option can be used to maximize resolution. Optimizing resolution of the target compound from all nearby impurities can be accomplished using the 3-COMPONENT option.

Method B: Optimized using PURITY; 3 COMPONENTS - spot 3 (target compound) and spots 2 & 4 (impurities)

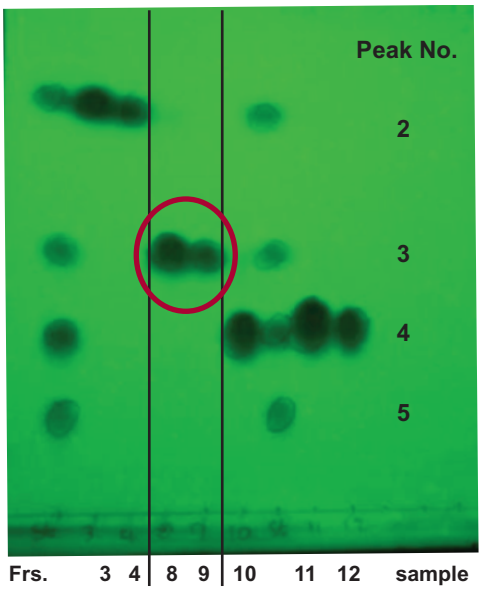
Gradient Method			
Step	**Min**	**Solvents**	**%B**
1	0.0	AB	10
2	1.6	AB	10
3	10.0	AB	26
4	6.1	AB	26
5	10.6	AB	26



Flash Purification of 5-component Crude mixture using Method B



Method B collected fractions



Method B Fraction Analysis by TLC

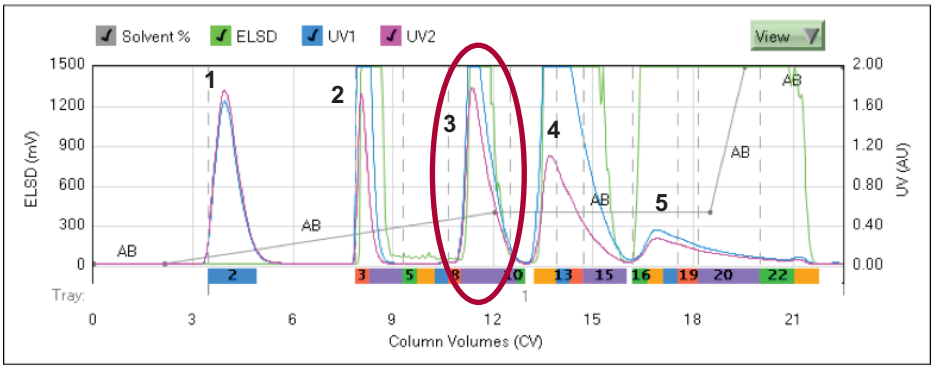
With the PURITY/3-COMPONENT method options, peak 3 (target) is fully resolved from neighboring impurities (peaks 2 & 4). The run is longer but baseline separation is fully achieved between peak 3 and peak 4, resulting in no loss of yield for peak 3. Based on the Rf input values and method choice, the Reveleris® Navigator™ held the gradient at 26% B, rather than increasing it to 36% B as it did in the SPEED option.

Method C

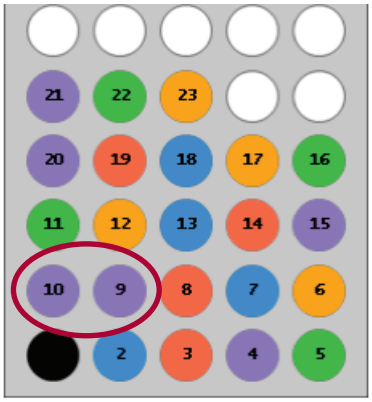
Maximum purity and yield can also be achieved using the PURITY/2-COMPONENT method option. Optimizing the resolution of the target compound from the closet eluting impurity can be accomplished using the 2-COMPONENT option. Increasing the gradient to 100% B at the end of the separation ensures that all components are eluted from the column.

Method C: Optimized using PURITY; 2 COMPONENTS - spot 3 (target compound) and spot 2 (impurity)

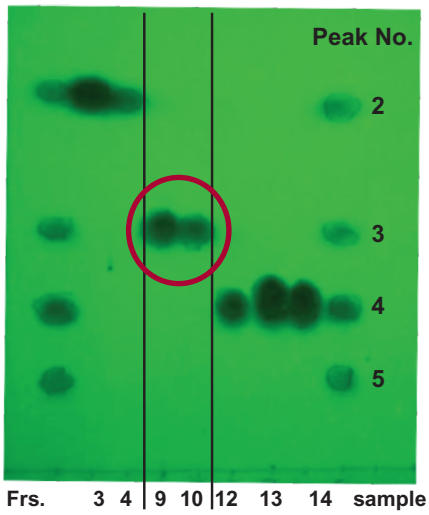
Gradient Method			
Step	**Min**	**Solvents**	**%B**
1	0.0	AB	10
2	2.1	AB	10
3	10.0	AB	26
4	6.5	AB	26
5	1.1	AB	100
6	3.0	AB	100



Flash Purification of 5-component Crude mixture using Method C



Method C collected fractions



Method C Fraction Analysis by TLC

With the PURITY/2-COMPONENT method option, peak 3 (target) is fully resolved from neighboring impurities (peaks 2 & 4). The run is the longest of the three because the gradient started at a lower %B and the option to continue the gradient to 100% at the end of the run was chosen to ensure all components were eluted from the cartridge. As a result, peak 3 (target) was retained longer and is better resolved from the earlier impurities (peaks 1 & 2). This strategy can be helpful in situations where higher loads may be needed.

Conclusion

As demonstrated above, the predictive power of the Reveleris® X2 Navigator™ software improves efficiency and productivity by simplifying method optimization, improving purity and recovery, and reducing run times and solvent use.

The Reveleris® X2 Navigator™ software can quickly determine the optimal method to meet the goals of the purification, preventing sample loss and rework.

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