



Reveleris® Navigator

What is Navigator?

- The *Navigator* is an advanced method optimizer designed to save you time and money with your purification challenges. It provides optimum gradient conditions based on input from other scouting methods like TLC and LC.
- Unlike traditional flash wizards, *Navigator's* advanced algorithm (patent pending) optimizes flash gradient conditions based on minimal starting input and then calculates the best conditions for either speed or purity.
- *Navigator* also optimizes methods not only around 2 component TLC data, but also on 3 component TLC data and RP LC data.

What Navigator is NOT?

- The *Navigator* is not a TLC wizard.
- Typical TLC wizards associated with flash systems input data from TLC (Rf values) and plot a gradient which typically has an isocratic hold around those two values in order to separate the values.
- There is no optimization that occurs with these wizards. Typically run times are longer with “wizards” or fractions may not be as pure.

Press the Navigator button to start the TLC wizard program

The screenshot shows the Grace software interface with the following settings:

- Method: gradient*
- Column: Generic Silica 80g
- Flow Rate: 60 mL/min
- Duration Units: min
- Equilibration: 6.3 min
- Run Length: 33.0 min
- Air Purge Time: 1.0 min
- Carrier: Iso-propanol
- Slope Detection:
- Sensitivity:
- Threshold Detection: ELSD 20 mV, UV 0.05 AU
- UV1 λ : 254 nm, UV2 λ : 280 nm
- Collect Peaks: Collect All, Collect None
- Per-Vial Volume: Maximum: 29.0 mL, Peak: 5.0 mL, Non-Peaks: 5.0 mL
- Solvents: A: Hexane, B: Ethyl acetate, C: <No solvent chosen>, D: <No solvent chosen>
- Estimated Solvent Use: A: 976 mL, B: 1402 mL, C: 0 mL, D: 0 mL, Total: 2378 mL
- Up To 476 Peak Vials, Up To 476 Non-Peak Vials

The **Navigator** button is highlighted in a red box. A dialog box titled **Navigator** is open, containing the following text:

Navigator is an advanced method optimizer designed to save you time and money with your purification challenges. It provides optimum gradient conditions based on input from other scouting methods like TLC and LC. If interested in purchasing or a trial of this feature, please contact your GRACE Sales Representative.

To activate the Navigator feature on your instrument, please enter your instrument-specific password.

Buttons: OK, Cancel

TLC Silica Tab

The TLC-Silica Tool uses TLC separation data to provide a recommended gradient for silica separations of 2 or 3 components.

To use this tool, separations of the sample components need to be obtained using two Reveleris® silica TLC plates with two different solvent concentrations. Calculate the R_f values of each spot by measuring the distance the spot moved and dividing this by the distance the solvent traveled.

Enter the information from these separations into the *TLC Conditions* section of the TLC-Silica Input Screen.

An exclusive feature of the *Navigator* is the ability to optimize the separation of 3 components when using the TLC-Silica tool. Selection of 2 components or 3 components using the radio buttons provides the correct number of input boxes for the R_f data.

Choose the solvents used from the dropdown lists. A is the weaker solvent, B is the stronger solvent. Above the plate depictions, enter the solvent concentrations used in the TLC separations. Along side of each plate depiction, enter the R_f values for each spot keeping the depicted order the same as that on the actual plate.

TLC Silica Tab

Navigator i

TLC - Silica | LC - C18 | LC Transfer

Enter TLC data and select Reveleris parameters, then press "Calculate" to generate a recommended gradient profile. Learn More

TLC Conditions:

Solvents:

A:

B:

2 Components
 3 Components

Lower %B: %

Higher %B: %

Component	Lower %B (20%)	Higher %B (30%)
Rf 1	0.69	0.75
Rf 2	0.57	0.68

Flash Conditions:

Column:

Flow Rate: mL/min

Optimize for:

Speed
 Purity

Continue gradient to 100%

Reset Close Calculate

Input Weak and Strong solvents used (Dropdown lists all solvents)

Suggested flow rate according to column size. User adjustable. Dropdown to include NP and RP columns

Input field to type or display spot value

Select either Speed, or Purity. Wash cartridge at end of run.



TLC Silica Output Screen.

Navigator i

TLC - Silica | LC - C18 | LC Transfer

Changing "Flash Conditions" will update table/graph. Press "Accept" to use the recommended gradient. Learn More

Min	% 2nd
0.0	3
0.6	3
1.4	9
2.5	9

The top portion of this screen shows the recommended gradient table and graph.

Flash Conditions:

Column: Reveleris™ Silica 12g

Flow Rate: 36 mL/min

Optimize for:

- Speed
- Purity

Continue gradient to 100%

Accept downloads the gradient into the Setup Screen after a quick check to determine if the correct cartridge is loaded in the instrument.

Close Go Back Accept

The bottom portion contains controls that can be adjusted without reentering the TLC data. Adjustments of these controls will change the table and graph.

Check for Correct Reveleris® Column

Navigator i

TLC - Silica LC - C18 LC Transfer

Changing "Flash Conditions" will update table/graph. Press "Accept" to use the recommended gradient. Learn More

Min	% 2nd
0.0	3
0.6	3
1.4	9
2.5	9

Choosing a mismatch cartridge will lead to a screen that requires the correct cartridge is installed before proceeding

Stop [X]

Please install the column specified below before pressing "Continue", otherwise the recommended gradient profile will change when a column is installed in the Method Setup screen.

Reveleris™ Silica 12g

Continue Cancel

Flash Conditions:

Column: Reveleris™ Silica 12g ▼

Flow Rate: 36 mL/min

Optimize for:

- Speed
- Purity

Continue gradient to 100%

Close Go Back Accept

Setup Screen with *Accepted* Gradient

File View Tools Help

Column: **Generic Silica 12g** Slope Detection

Flow Rate: mL/min Sensitivity:

Duration Units: min CV

Equilibration: min

Run Length: min

Air Purge Time: min

Threshold Detection:

ELSD mV

UV AU

ELSD UV1 λ : nm

Carrier: **Iso-propanol** UV2 λ : nm

Collect Peaks

Collect All

Collect None

Per-Vial Volume:

Maximum: mL

Peak: mL

Non-Peaks: mL

(18x150mm) (18x150mm)

Solvents:

A: **Hexane**

B: **Ethyl acetate**

C: **<No solvent chosen>**

D: **<No solvent chosen>**

Estimated Solvent Use:

A: 272 mL

B: 15 mL

C: 0 mL

D: 0 mL

Total: 287 mL

Up To 57 Peak Vials

Up To 57 Non-Peak Vials

Table Navigator Edit

Solvent %

Time (min)

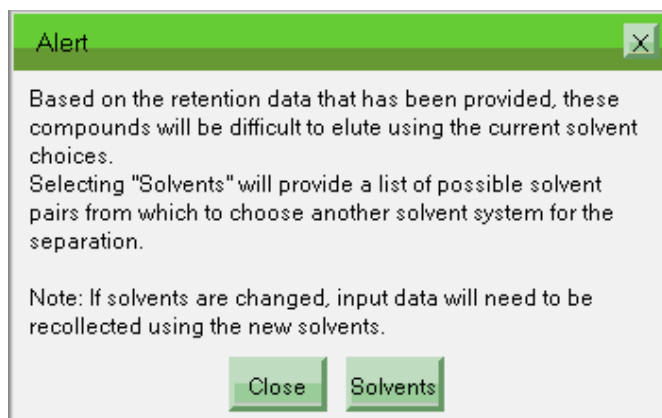
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Navigator helps guide for further optimization.

After pressing the *Calculate* button, the Navigator performs several checks before providing a gradient condition for optimum separation. If elution issues exist, several screens or messages will be displayed to describe the issue and recommended solution.

In cases where it may difficult to elute the compounds of interest, an *Alert* screen will be displayed. In this situation, it is strongly recommended that a new solvent pair is chosen and rerun the TLC separation

Press *Close* to remove the *Alert* or press *Solvents* to view a list of possible solvents that may be tried. Choosing a pair with higher solvent strength should allow elution of the components. New TLC data will need to be generated with the chosen pair.

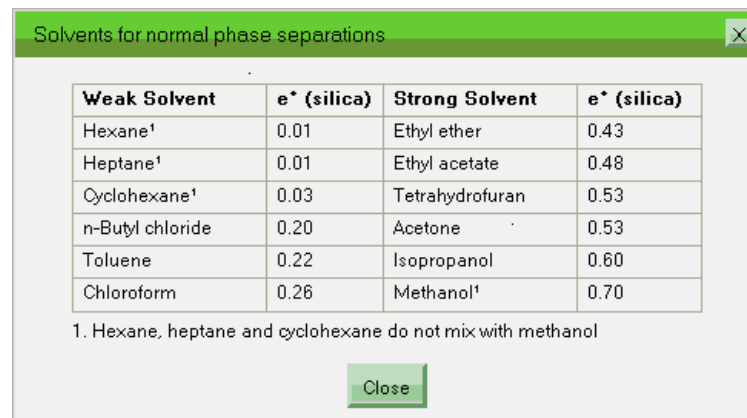


Alert

Based on the retention data that has been provided, these compounds will be difficult to elute using the current solvent choices. Selecting "Solvents" will provide a list of possible solvent pairs from which to choose another solvent system for the separation.

Note: If solvents are changed, input data will need to be recollected using the new solvents.

[Close](#) [Solvents](#)



Solvents for normal phase separations

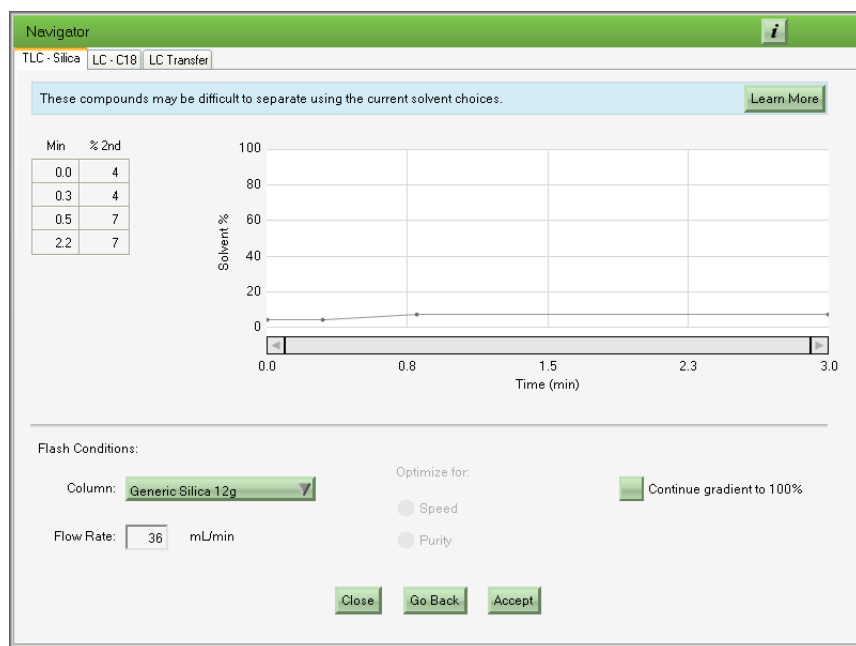
Weak Solvent	e* (silica)	Strong Solvent	e* (silica)
Hexane ¹	0.01	Ethyl ether	0.43
Heptane ¹	0.01	Ethyl acetate	0.48
Cyclohexane ¹	0.03	Tetrahydrofuran	0.53
n-Butyl chloride	0.20	Acetone	0.53
Toluene	0.22	Isopropanol	0.60
Chloroform	0.26	Methanol ¹	0.70

1. Hexane, heptane and cyclohexane do not mix with methanol

[Close](#)

Navigator predicts and guides the user for optimal results.

Another issue is poor separation of components. If the *Navigator* detects that there may be resolution problems, a message will be displayed in the blue information bar. In some cases the *Speed* and *Purity* buttons will also be disabled. Pressing *Learn More* will give an Alert. Press *Accept* to transfer the recommended gradient to set-up screen or chose a different solvent pair as above and rerun the TLC with these solvents.



The Alert dialog box contains the following text:

Based on the retention data that has been provided, these compounds may be difficult to separate using the current solvent choices. Selecting "Solvents" will provide a list of possible solvent pairs from which to choose another solvent system for the separation. In some instances the nature of the alert will not allow selection of "Speed" and "Purity" and these buttons will be disabled.

Note: If solvents are changed, input data will need to be recollected using the new solvents.

Buttons: "Close" and "Solvents"

LC C18 Tab

The LC-C18 Tool uses isocratic HPLC separation data to provide a recommended gradient for reversed phase separations of 2 components.

To use this tool, separations of the sample components need to be obtained in two isocratic runs on a Reveleris® C18 HPLC column with different mobile phase solvent concentrations. Obtain the retention times (t1 and t2) of the components from the chromatograms.

The void time (t0) may be measured using a void marker in the sample mix or a default value shown on the screen may be used.

Enter the information from these separations into the HPLC Conditions section of the screen.

Select the HPLC column from the dropdown list and enter the HPLC flow rate used. The default t0 value is calculated each time the HPLC column is selected from the dropdown list or each time the HPLC flow rate value is changed. Choose the solvents used from the dropdown lists. A is the weaker solvent, B the stronger solvent. Above the chromatogram depictions, enter the solvent concentrations used in the HPLC separations.

Enter the retention time (t1 and t2) for each separation under each chromatogram. To override the default t0, enter a measured t0 into the corresponding boxes.

LC C18 Tab

Select Reveleris HPLC column used. (Dropdown lists all columns)

Navigator i

TLC - Silica | **LC - C18** | LC Transfer

Enter HPLC Data and select Reveleris parameters. Press "Calculate" to generate recommended gradient profile. Learn More

HPLC Conditions:

Column: **Reveleris LC-C18 (150 x 4.6mm)**

Flow Rate: mL/min

Solvents:

A: **Water**

B: **Methanol**

Lower %B

t0 t1 t2

Higher %B

t0 t1 t2

Flash Conditions:

Column: **Reveleris™ C18 12g**

Flow Rate: mL/min

Optimize for:

Speed

Purity

Continue gradient to 100%

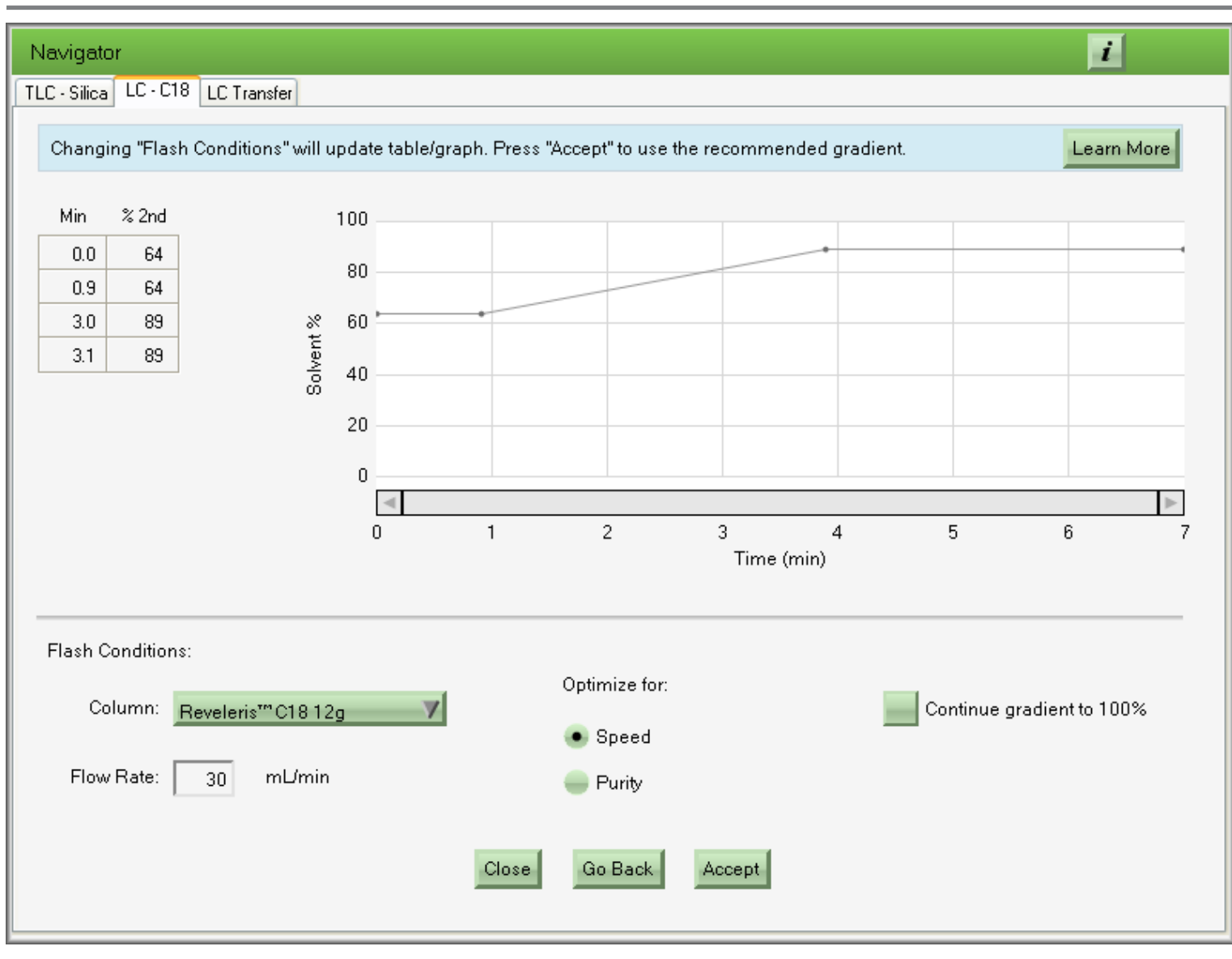
Set conditions used

Set results from isocratic runs.

The bottom portion contains controls similar to that in TLC. Select proper settings

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C18 Output Screen.



C18 Warnings and Prompts

Check for Correct Column

Solvent match based on conditions

Weak Solvent	e* (C18)	Strong Solvent	e* (C18)
Water		Methanol	1.0
		Acetonitrile	3.1
		Tetrahydrofuran	3.7
		Isopropanol	8.3
		Acetone	8.8
		1,4-dioxane	11.7

Alternate Solvent Suggestions

LC Transfer Tab

The LC-Transfer tool converts any HPLC gradient into a Reveleris® Flash Chromatography gradient.

To use this tool, input information from an HPLC gradient that is suitable to be transferred into a flash chromatography into the LC-Transfer Input Screen

LC Transfer Tab

Navigator i

TLC - Silica | LC - C18 | **LC Transfer**

Enter HPLC data and select Reveleris parameters, then press "Calculate" to generate a recommended gradient profile. [Learn More](#)

HPLC Conditions:

Column: 150 x 4.6 mm ▼

Flow Rate: 1 mL/min

HPLC t₀: 1.62 min

Higher %B 90

Lower %B 10

t1 1 t2 11 t3 13

Flash Conditions:

Column: Generic 12g ▼

Flow Rate: 36 mL/min

[Reset](#) [Close](#) [Calculate](#)

LC Transfer Prompts

Check for Correct Reveleris® Column

Navigator

TLC - Silica | LC - C18 | LC Transfer

Changing "Flash Conditions" will update table/graph. Press "Accept" to use the recommended gradient. [Learn More](#)

Min	% 2nd
0.0	10
0.3	10
2.9	90
0.3	90

Solvent %

100
80
60

2.6 3.5

Stop

Please install the column specified below before pressing "Continue", otherwise the recommended gradient profile will change when a column is installed in the Method Setup screen.

Reveleris™ Silica 12g

Continue Cancel

Flash Conditions:

Column: Reveleris™ Silica 12g

Flow Rate: 36 mL/min

Close Go Back Accept

If the Reveleris® cartridge installed on the instrument matches the Reveleris® cartridge listed in the *Column* field, pressing the *Accept* button transfers the gradient condition into the Setup Screen. Otherwise, it will lead to a screen that requires the correct cartridge is installed before proceeding.

Check for Correct Generic Column

Navigator

TLC - Silica | LC - C18 | LC Transfer

Changing "Flash Conditions" will update table/graph. Press "Accept" to use the recommended gradient. [Learn More](#)

Min	% 2nd
0.0	10
0.3	10
2.9	90
0.3	90

Solvent %

100
80
60

2.6 3.5

Warning

Please confirm the column specified below is installed before pressing "Continue", otherwise the recommended gradient profile will change when a column is installed in the Method Setup screen.

Generic Silica 12g

Continue Cancel

Flash Conditions:

Column: Generic Silica 12g

Flow Rate: 36 mL/min

Close Go Back Accept

If a Generic cartridge is chosen in the *Column* field, pressing the *Accept* button leads to a warning screen. Please confirm that the cartridge specified in the *Column* field is installed on the instrument. If not, install the specified column. Then, press *Continue* to transfer the gradient conditions to the Setup Screen.

Example of Results – Case Study

2 Component Normal Phase:

Experimental:

Equipment:

TLC tank
10 x 10cm Reveleris Si plates
Competitor's software system

Reveleris flash instrument Part No 5148513
Cartridge: Reveleris Silica 12g Part No 5146131
Mobile phases: A: Hexane, B: Ethyl acetate
Sample Mix: alpha tocopherol, delta tocopherol in hexane

Traditional Optimization Procedure:

Method development is conducted by repeatedly spotting TLC plates with sample mixture and developing the plate in a solvent chamber containing a enough solvent to raise to a 1 cm height from the bottom with a mix of a solvent pair. The plate is allowed to sit in the chamber and wick up isocratic mix of solvent until it nears the top of the plate.. Optimization takes place by the chemist selecting TLC plate conditions that best separate the components of interest (visible by UV light irradiation or chemical staining) from 1-3 near eluting components or impurities. The resulting component R_fs (measured as height of elution) are used as software input values for gradient predictor software required to generate a flash gradient method conditions.

Resulting Gradient:

Flow Rate: 36 ml/min

Time (min)	%B
0	0
4.2	0
11	100
3	100
0	0
1	0

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Example of Results – Case Study

The RevealX™ Operating System Procedure:

Two isocratic TLC runs are made with the sample mixture using the same solvent pair. The Rf values are entered into the Reveleris® Navigator method predictor feature which automatically generates an ideally optimized method of choice. One of two method options is selected: Either a “Purity” method designed for maximizing resolution or a “Speed” method for minimizing run time/solvent use. Method optimization is automatically performed by the software system.

Gradient Outputs:

Purity Method:

Flow Rate: 36 ml/min

Time (min) %B

0 3

0.6 3

4.7 9

2.8 9

Speed Method:

Flow Rate: 36 ml/min

Time (min) %B

0 3

0.6 3

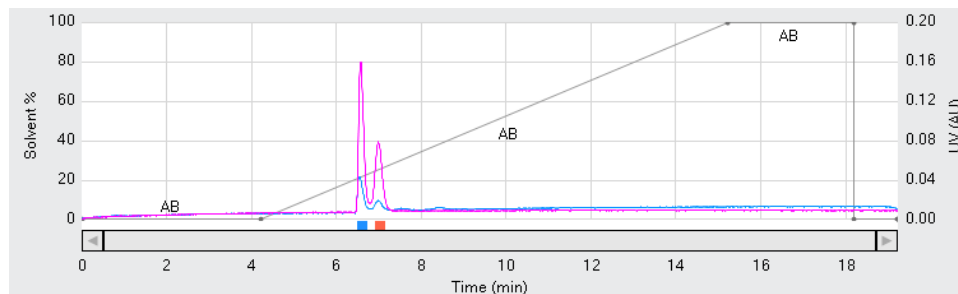
1.4 9

2.5 9

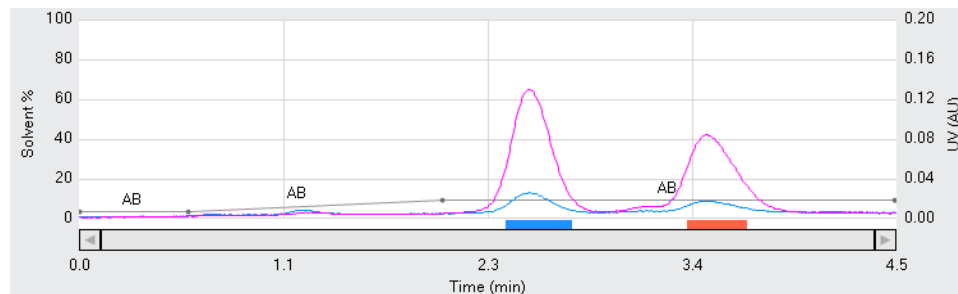
Example of Results – Case Study

Results: 2 Component NP		Reveleris Purity	Reveleris Speed	Traditional Method
Method Development	Time (min)	52	52	78
	Solvent Used (ml)	50	50	75
Method Performance	Resolution	2.94	2.04	1.50
	Time (min)	8.1	4.5	19.2
	Solvent Used (ml)	292	162	691
	Total Time (min)	60.1	56.5	97.2
	Total Solvent Used (ml)	342	212	766

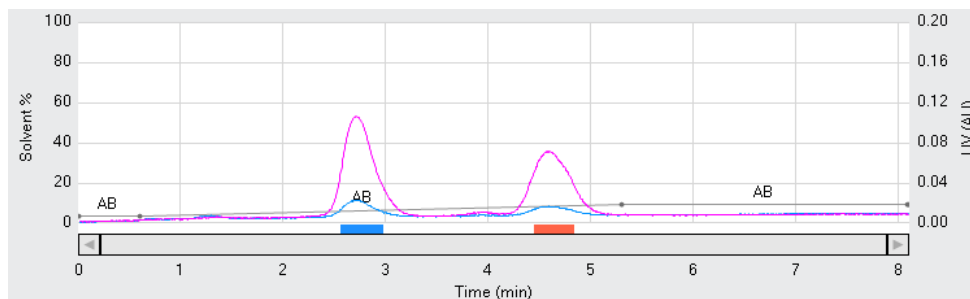
Example of Results – Case Study



Traditional System Method



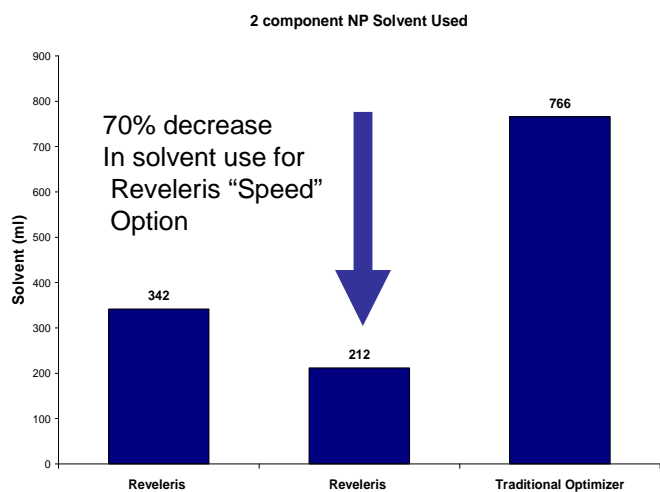
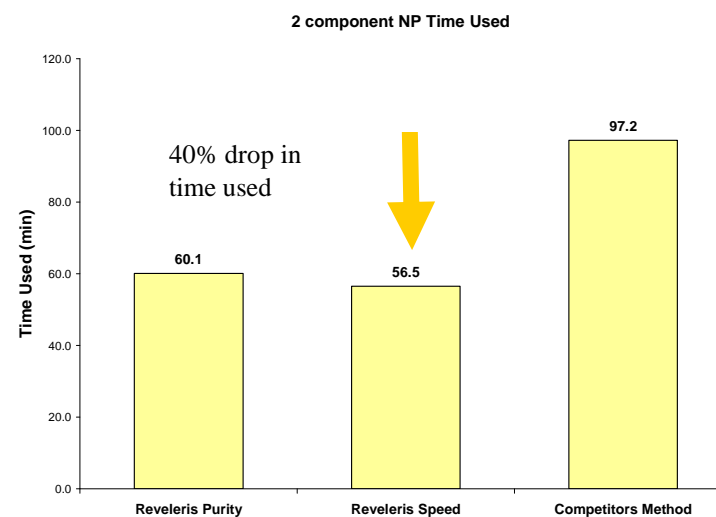
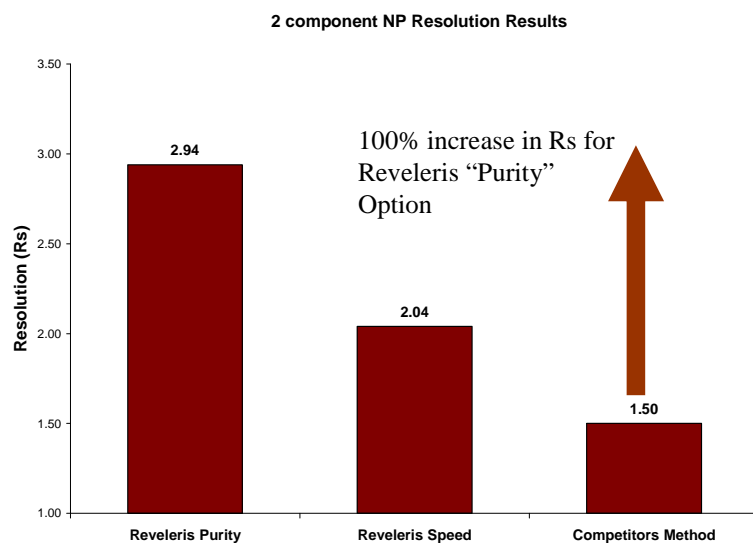
Reveleris Speed



Reveleris Purity



Example of Results – Case Study



Conclusion

The ReveaIX Operating System Navigator feature was successfully employed to guide flash purification method optimization for quality, speed, or reduced costs. It is now possible to easily improve methods using “Speed” or “Purity” options within the method predictor software using Reveleris® Navigator.

2-Component NP separation was optimized for “Purity” resulting in twice the resolution to the traditional approach. The “Speed” method uses 70% less solvent and 40% less total time. The 3-Component NP separation employing the Reveleris® Navigator method resulted in 40% higher resolution in peaks 1 & 2 and uses 60% less solvent than the traditional method and an improvement in total time saved by 40%.

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