Automating Gradient Method Development in Flash Chromatography For Greater Productivity and Minimizing Solvent Use

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Abstract

Productivity demands in today's laboratories require the chemist to minimize time spent purifying compounds. Faster, 'greener' flash chromatography methods increase throughput, improve productivity, save solvent, and reduce operating costs. Meeting these goals requires gradient methods that deliver the required resolution in the fastest possible time. The RevealX™ Operating System of the Reveleris® flash chromatography system automatically generates gradient profiles using only two chromatographic separations (TLC or HPLC) as inputs and provides methods for both normal phase and reversed phase systems. The chemist can choose a gradient profile based on either highest purity or fastest speed.

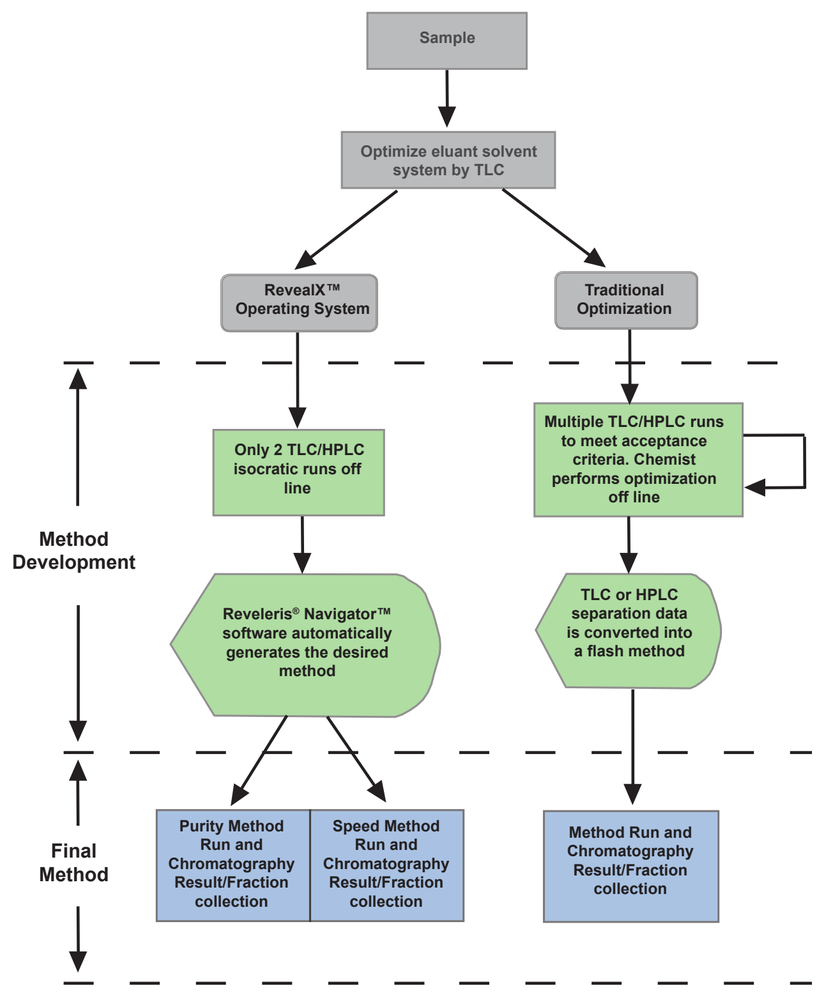
In this work both of these gradient profiles were compared with traditional gradient development routes for normal and reversed phase chromatography examples. This work shows a productivity gain, when using this operating system for gradient method development, by increasing chromatographic resolution, reducing time spent optimizing the separation and reducing solvent used compared to traditional methods.

Introduction

This paper compares the productivity of the RevealX™ Operating System on a Reveleris® flash chromatography system with traditional flash method optimization. Flash purifications are improved for quality, speed, and cost easily by the control capability of the "Navigator" feature of the operating system.

Productivity of the final flash purification method was determined by measuring resolution for the chromatographic compound pairs, time savings, and solvent used to run the method.

Experiments were conducted to evaluate the following separations and mixture types: a two component normal phase (NP), three component normal phase (NP) and two component reversed-phase (RP) separation. All chromatography comparisons were made using the same instrument and flash cartridge or HPLC column. An overview of two development pathways and overall process is shown below.



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 enough solvent to raise to a 1cm height from the bottom with a mix of a solvent pair. The plate is allowed to sit in the chamber and wick up an 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 Rfs (measured as height of elution) are used as software input values for gradient predictor software required to generate flash gradient method conditions.

Gradient: **Flow Rate:** 36mL/min

Time:	0	4.2	15.2	18.2	18.2	19.2
%B:	0	0	100	100	0	0

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.

Gradients:

Purity Method:
Flow Rate: 36mL/min

Time:	0	0.6	5.3	8.1
%B:	3	3	9	9

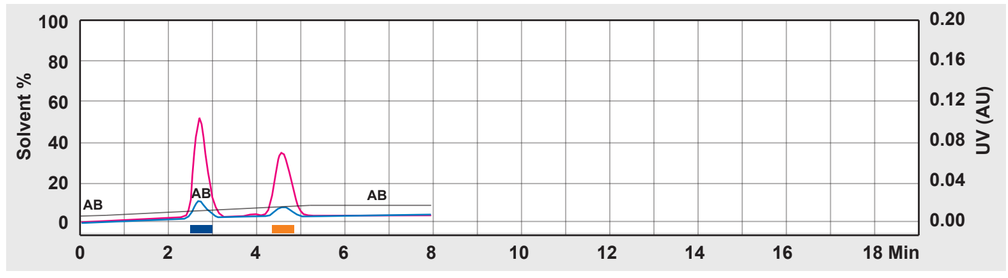
Speed Method:
Flow Rate: 36mL/min

Time:	0	0.6	2.0	4.5
%B:	3	3	9	9

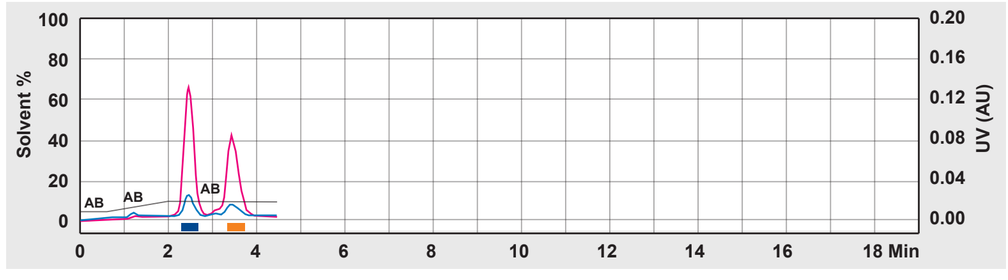
Results: 2-Component Normal Phase

		Reveleris® System Purity Method	Reveleris® System or Flash System Speed Method	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**

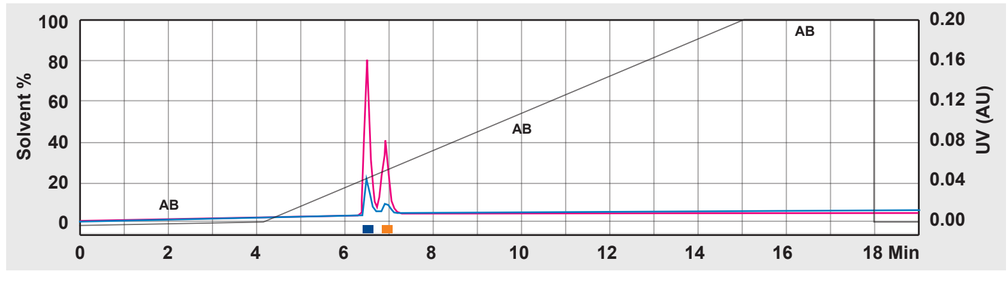
Reveleris® Flash Chromatography System Purity Method



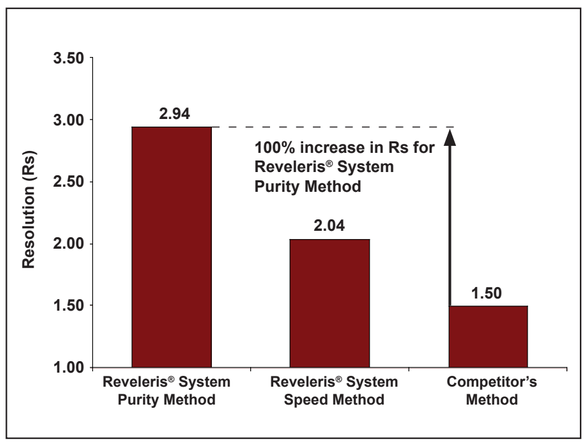
Reveleris® System Speed Method



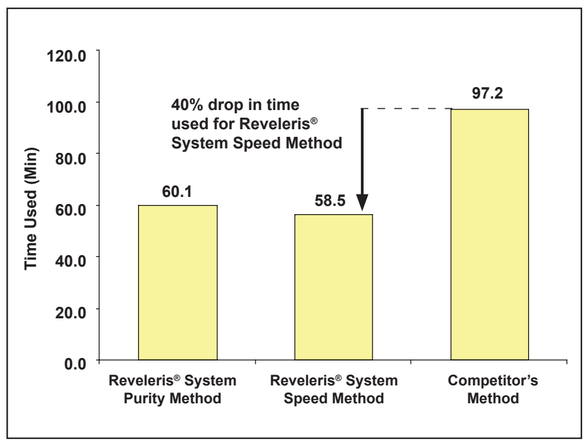
Competitor's Method



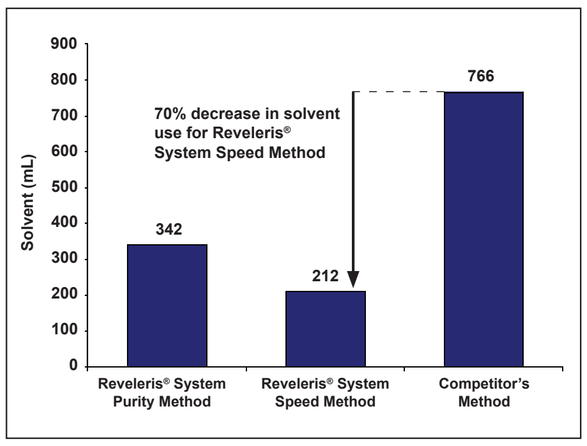
2-Component NP Resolution Results



2-Component NP TIme Used



2-Component NP Solvent Used



So we see that the competitor method has the lowest resolution, longest time, and uses the most solvent. By using the RevealX™ operating system and choosing speed as the rate limiting factor, the baseline resolution between the peaks is still achieved and the minimum amount of solvent is used with the shortest time.

3-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, methyl paraben in hexane/ethyl acetate

Traditional Optimization Procedure:

Method predictor software for three components is not available. The method predictor software previously used in 2-component NP was employed to generate a flash method gradient based on the two Rf values having the least difference.

Gradient Method: **Flow Rate:** 36 mL/min

Time:	0	3.2	3.2	4.2	15.2	18.2	18.2	19.2
%B:	0	0	0	0	100	100	0	0

The RevealX™ Operating System Procedure:

Two isocratic TLC plate tests were conducted, instead of the usual numerous tests, with the three component sample mix. Three Rf values are entered into the Reveleris® Navigator™ method predictor feature including the component of interest and two of it's most closely eluting impurities. An optimized method was automatically generated and parameters loaded in preparation in advance of the flash purification run. The method optimizes the separation for all three components for maximum resolution, least time and minimal solvent use.

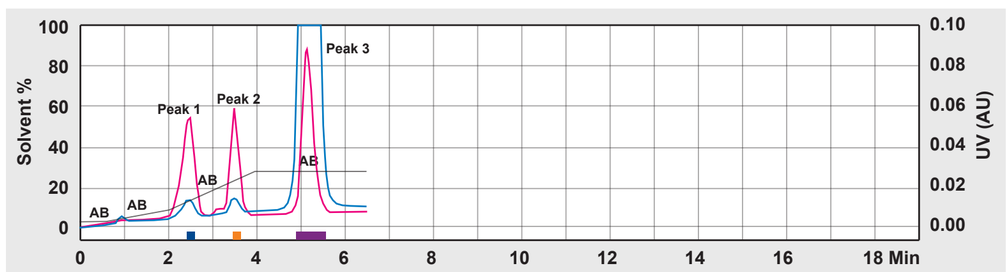
Gradient Method: **Flow Rate:** 36mL/min

Time:	0	0.6	2.0	3.9	6.4
%B:	3	3	9	27	27

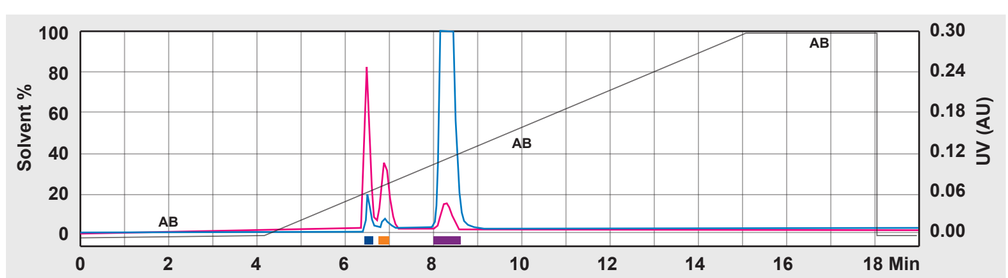
Results: 3-Component Normal Phase

		Reveleris® System Method	Traditional Method
Method Development	Time (min)	52	78
	Solvent Used (mL)	50	75
Method Performance	Resolution peak 1 & 2	2.08	1.46
	Resolution peak 2 & 3	3.5	3.4
	Time (min)	6.4	19.2
	Solvent Used (mL)	230	691
	Total Time (min)	**58.4**	**97.2**
	Total Solvent Used (mL)	**280**	**766**

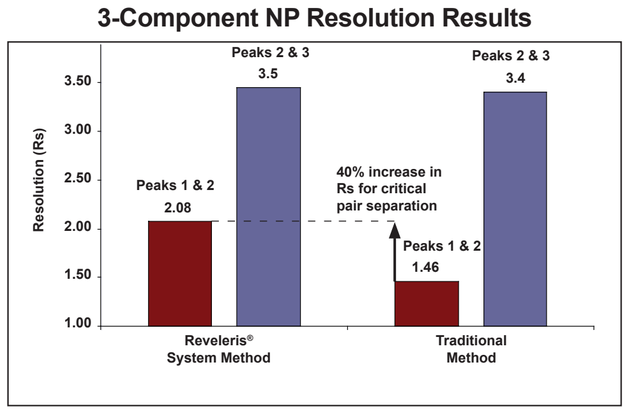
Reveleris® System Method



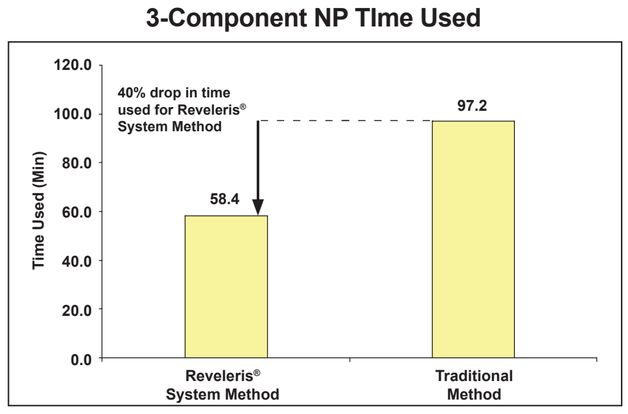
Traditional Method



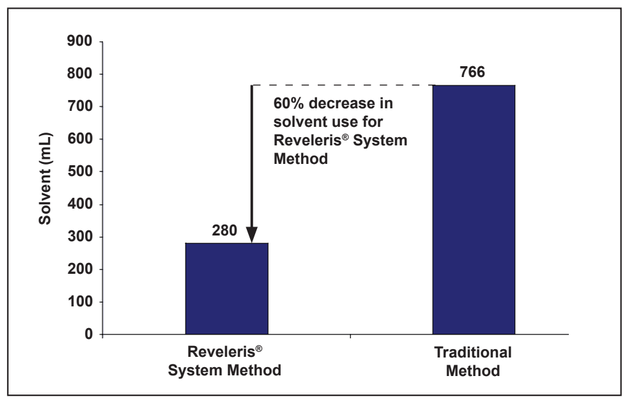
3-Component NP Resolution Results



3-Component NP TIme Used



3-Component NP Solvent Used



Similarly with this example, the competitor method has the lowest resolution, particularly between peaks 1 and 2 , as well as using the most solvent and taking the longest time.

2-Component Reversed-Phase

Experimental:

Equipment:
HPLC Instrument: Agilent 1100
HPLC Column: Reveleris® C18 4.6 x 100mm
Reveleris® flash instrument Part No **5148513**
Cartridge: Reveleris® C18 12g Part No **5152103**
Mobile Phase: A: DI water, **B:** Methanol
Sample Mix: 1% benzoic acid 0.5% caffeine in 30% MeOH 70% DI water

Traditional Optimization Procedure:

Gradient method development is performed by the chemist on an HPLC system using an analytical column. Multiple runs are made to optimize the gradient through a manual iterative approach resulting in several analysis runs. This HPLC method is then converted to a flash gradient method manually, by calculating volumes and scaling up accordingly.

Gradient:
Flow Rate: 30mL/min

Time:	0	0.5	5.8
%B:	40	40	65

The RevealX™ Operating System Procedure:

Two scouting runs were performed, versus the usual unknown iterative number of runs, at different isocratic mobile phase conditions using a Reveleris® HPLC analytical column using the same solvent pair. Retention times of the component of interest and its nearest impurity were entered into the Reveleris® Navigator™ method predictor software to automatically generate two optimized methods. A “Purity” method to maximize resolution/target compound recovery and a “Speed” method to minimize time/solvent are used.

Gradients:

Purity Method:
Flow Rate: 30mL/min

Time:	0	0.9	6.9	10.6
%B:	38	38	55	55

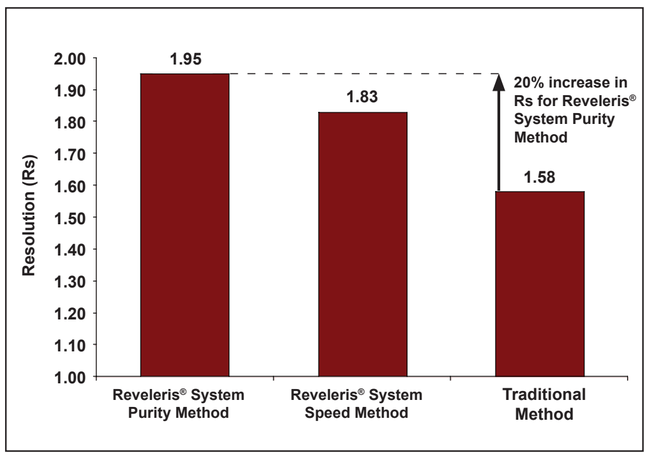
Speed Method:
Flow Rate: 30mL/min

Time:	0	0.9	2.7	6.0
%B:	38	38	55	55

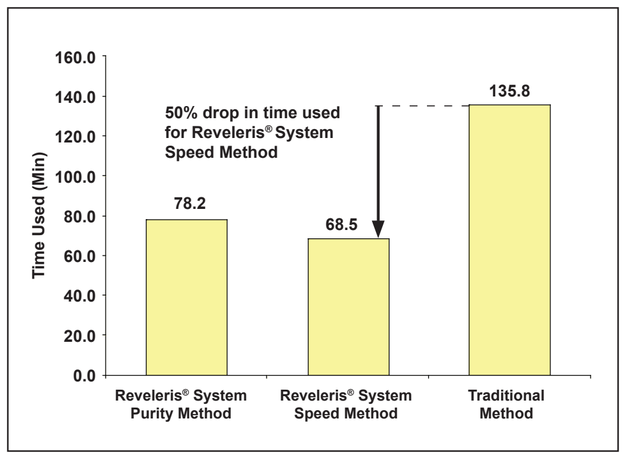
Results: 2-Component Reversed-Phase

		Reveleris® System Purity Method	Reveleris® System Speed Method	Traditional Method
Method Development	Time (min)	67.6	62.5	130
	Solvent Used (mL)	50	50	125
Method Performance	Resolution	1.95	1.83	1.58
	Time (min)	10.6	6.0	5.8
	Solvent Used (mL)	318	180	174
	Total Time (min)	**78.2**	**68.5**	**135.8**
	Total Solvent Used (mL)	**368**	**230**	**299**

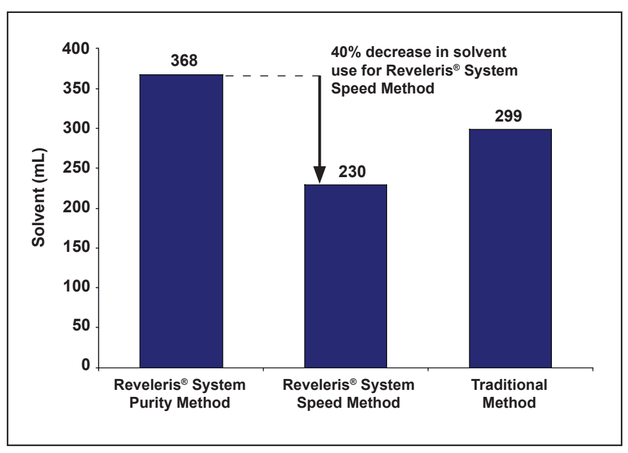
2-Component RP Resolution Results



2-Component RP TIme Used



2-Component RP Solvent Used



The RevealX™ operating system is equally valuable for time and solvent savings with reversed-phase separations. The data again shows increased resolution with lower solvent consumption and reduced time.

Conclusion

The RevealX™ 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, uses 60% less solvent than the traditional method and an improvement in total time saved by 40%. The 3 Component NP Reveleris® Navigator™ is the only method to solve 3 component flash separations automatically, which can take multiple calculations in competitive software.

The 2-Component RP Reveleris® Navigator™ “Purity” and “Speed” optimization resulted in 40% solvent and 50% total time saved with 20% increased resolution compared to a manual iteratively development method. This is the only known RP optimizer software to automatically generate flash methods from LC data.

The traditional methods require repeated experimentation until the method is optimized or the Rf values meet some set criteria; some chromatographic knowledge to perform this is required. The Reveleris® Navigator™ automatically performs this optimization in all 3 examples.

An additional feature of the Reveleris® Navigator™ is to automatically transfer HPLC reversed or normal phase method details into a Flash method.

The RevealX™ Operating System allows a relatively inexperienced chromatographer to develop methods that separate target compounds with increased purity (resolution), with less solvent (time) and to do this with less development steps compared to traditional methods.