

## Overview

- Multi-column analytical screening is a common high throughput approach for accelerating method development for chromatographic purifications.
- At Pfizer-La Jolla, supercritical fluid (SFC) and high performance liquid (HPLC) chromatography are used orthogonally to enable chromatographers to utilize either technique to maximize successful purifications.
- Data analysis time and complexity of chromatographic data are bottlenecks to purification workflow efficiency
- Analytical Studio Professional – Compound QC enables automated data processing, selective scoring, and visualization of acquired SFC/MS and LC/MS data and selects appropriate methods using advanced data evaluation criteria.

## Introduction

SFC and HPLC techniques are utilized to maximize the chance of obtaining ideal separation conditions for medicinal chemistry compounds. The number of method conditions and thus the time required an expert to review each piece of acquired data is extremely time consuming. Chromatographic challenges may also be present such as: multiple component-of-interest (target) peaks within an analytical screening run, which hinders the identification of the intended target; co-elution and incomplete resolution of target and impurities; and chromatographic suitability factors such as retention times and peak shapes. All of these are important considerations when selecting appropriate methods for purification and, therefore, are bottlenecks to an automated approach. To remove the complexity and time restraints associated with data analysis, an automated method selection package was implemented to analyze, review, and select the best SFC and HPLC screening conditions. Customized software scoring algorithms were created. Samples that are submitted to the analytical LC/MS and SFC/MS screens, are processed to automatically select the run with the highest method score as the “best” separation method. This approach improves overall workflow efficiency and enables chromatographers to focus more on purification-related tasks.

## Methods

**SFC/MS:** Mobile phase consists of 1-CO<sub>2</sub>; 2-Methanol. Flow rate 3.5 mL/min, run time 5 mins, APCI (+) ionization.

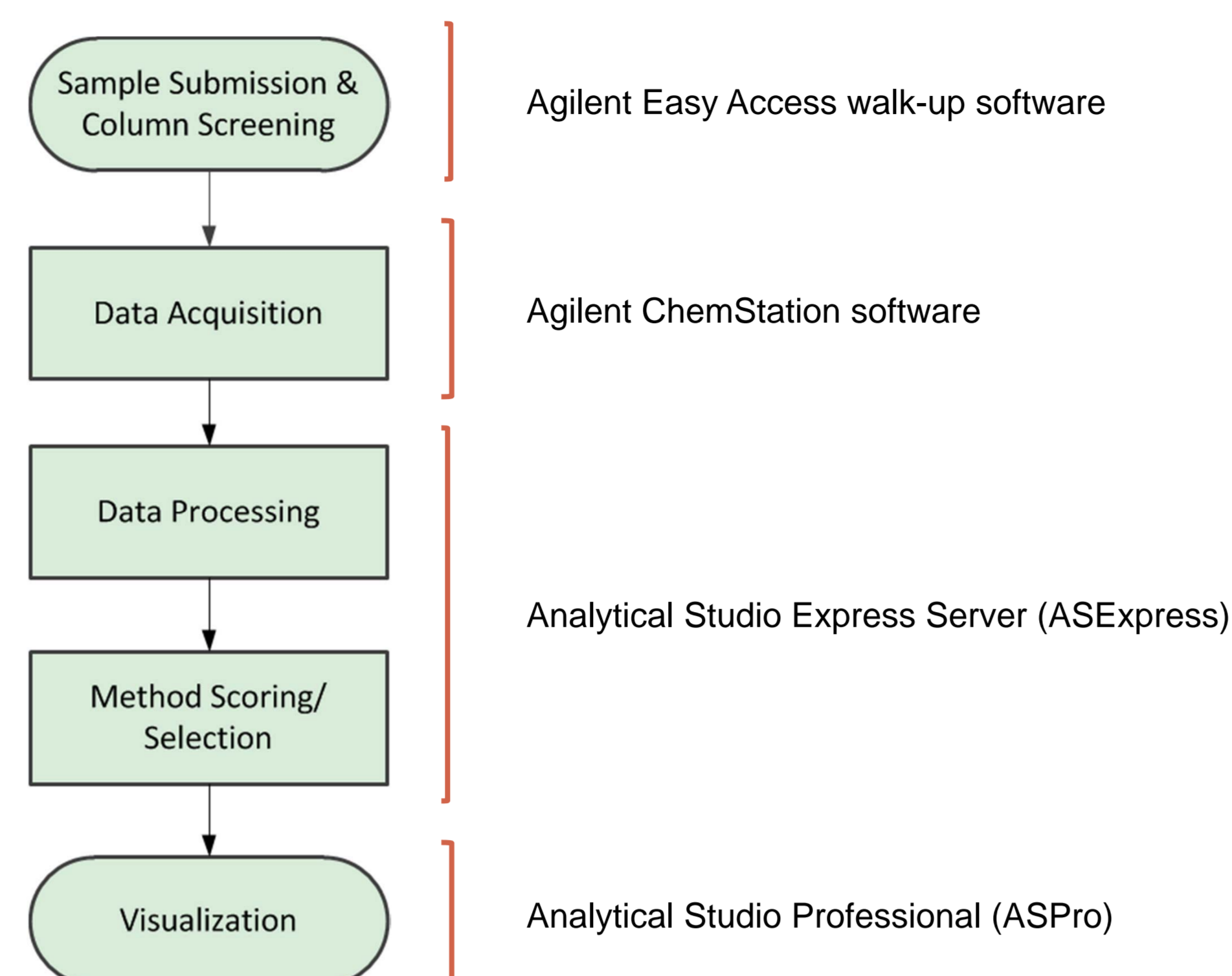
Method	Column	Gradient Conditions
A1	ZymorSPHER Pyr/Diol, 150x4.6mm, 5u, 100A	5-50% (2) in 3.4 mins; hold for 0.6 mins
A2	ZymorSPHER HADP, 150x4.6mm, 5u, 100A	7.5-50% (2) in 3.4 mins; hold for 0.6 mins
A3	ZymorSPHER HAP, 150x4.6mm, 5u, 60A	7.5-50% (2) in 3.4 mins; hold for 0.6 mins
A4	ZymorSPHER C8/PE, 150x4.6mm, 5u, 100A	5-50% (2) in 3.4 mins; hold for 0.6 mins
A5	ZymorSPHER Diol/Monol, 150x4.6mm, 5u, 100A	5-50% (2) in 3.4 mins; hold for 0.6 mins

**LC/MS:** Mobile Phase for B1, 1-Acetonitrile; 2-10mM NH<sub>4</sub>OAc in H<sub>2</sub>O. For B2, 2-0.05% TFA in H<sub>2</sub>O. Flow rate 2.25 mL/min, run time 4.5 mins., APCI (+) ionization.

Method	Column	Gradient Conditions
B1	Phenomenex Gemini C18, 50x4.6mm, 5u 110A	0-100 (1) in 3.0 mins; hold for 0.75 mins
B2	Phenomenex Gemini C18, 50x4.6mm, 5u 110A	0-100 (1) in 3.0 mins; hold for 0.75 mins

Operating software includes ChemStation B.03.01 with Easy Access walk-up shell. The automated software utilized Analytical Studio Express and Analytical Studio Professional from Virscidian with customized data interpretation and results visualization.

## Automated Solution Workflow



## Method Selection Criteria

Separation Value	Determines degree of co-elution and separation of target and impurities from reconstructed EIC composite chromatograms (1) those m/z associated with the target (i.e. isotope ions, fragments, adducts, etc) (2) unknowns; scored as 0 or 10. e.g. chromatograms with no co-elution of impurities with target peak are assigned 10.
Asymmetry & Tailing	Determine best target peak shape; independently calculated from Total Wavelength Chromatogram (TWC) at half height; 0-10 scale
Pre/Post Resolution	Resolution of neighboring peaks in the TWC; 0-10 scale. TWC channel is used to match a UV-triggered purification approach.
Multiple Target Peaks	Determines the presence of multiple target peaks with same m/z; 0-10 scale, with 10 indicating the presence of only 1 target.

## Method Selection Solution Highlights

### Analytical Studio Professional View

Row A: SFC/MS  
Row B: LC/MS

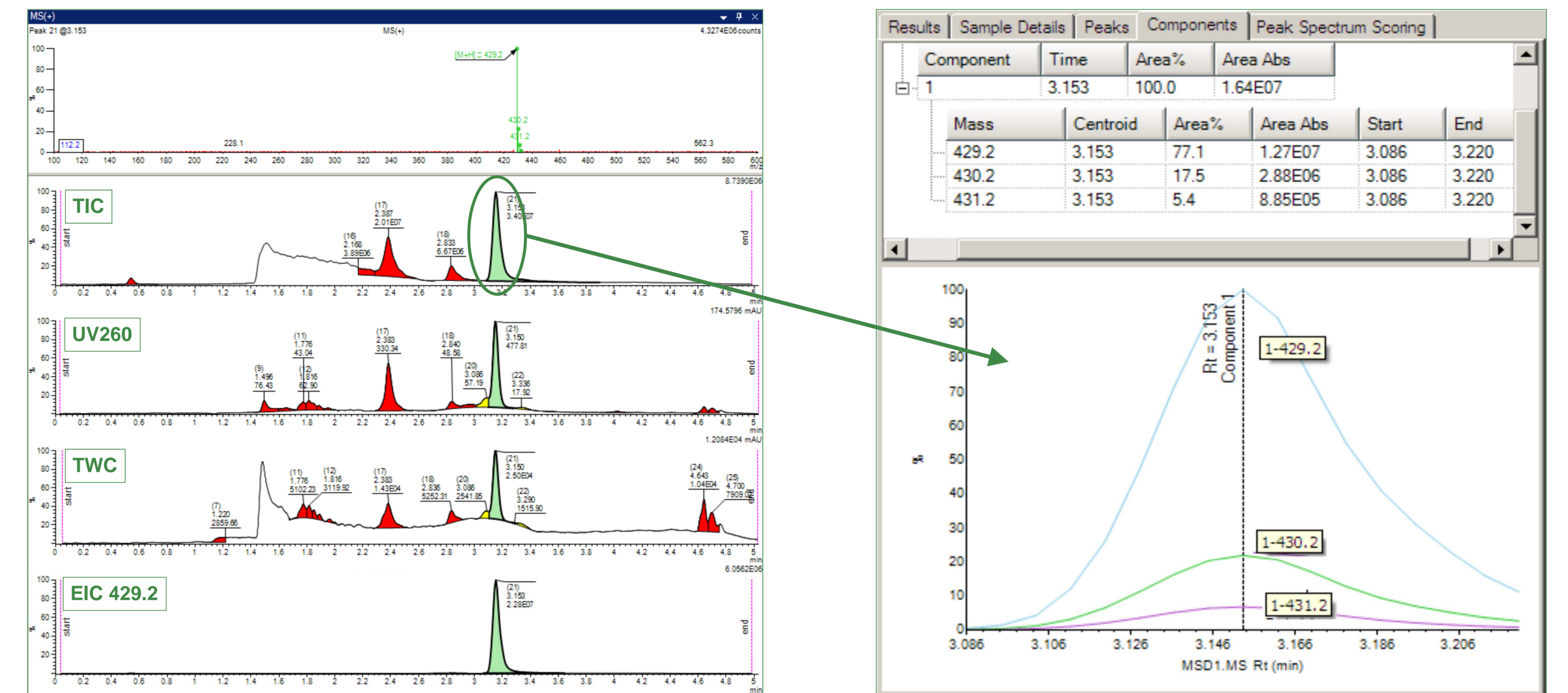
ASExpress selected “Best” SFC method  
ASExpress selected “Best” LC method

Well A5 detail summary

Well A5 scoring summary

Rank	Expression Name	Result	Expression
1	MethodScore	40.00	VarSeparationValue+Var
2	SeparationValue	10.00	VarSeparationValue
3	Tailing	4.000	VarTailing
4	PreRes	5.000	VarPreRes
5	PostRes	10.00	VarPostRes
6	MultiCOI	10.00	VarMultiCOI
7	Asym	1.000	VarAsym

## Well A5 Results – Analytical Studio Professional view



User-defined viewable data set. Green peak is targeted target.

Componentization of target peak shows no impurities, only adducts, which confirms peak purity.

## Scoring Summary of All Methods

Displayed Sample	Location	MethodScore	SeparationValue	Tailing	PreRes	PostRes	MultiCOI	Asym
110511-DIOL_M	A5	40.000	10.000	4.000	5.000	10.000	10.000	1.000
110511-PYR_DIO	A1	39.000	10.000	9.000	5.000	10.000	5.000	0.000
110511-HAP_SC	A3	38.000	10.000	8.000	10.000	5.000	5.000	0.000
110511-AMAC_P	B1	38.000	10.000	3.000	10.000	10.000	5.000	0.000
110511-HADP_S	A2	28.000	0.000	3.000	10.000	10.000	5.000	0.000
110511-C8_PE_S	A4	27.000	0.000	7.000	5.000	5.000	10.000	0.000
110511-TFA_PR	B2	20.000	0.000	8.000	10.000	5.000	5.000	0.000

Visualization of composite chromatograms for all methods enables quick detection of co-eluting peaks within a method. Wells A2, A4, A5 and B2 all show the presence of co-eluting unknowns with the target peak. The ratio of impurity peak area to target peak area of Method A5 fell below threshold, while A2, A4 and B2 were above the threshold. As a result, A5 received a Separation Value of 10, while A2, A4 and B2 received scores of 0.

Method A5 showed the presence of multiple, separated target peaks (1 major, 1 minor), as did A4. Multiple target peaks were not detected in all other methods; therefore these only scored 5 for the Multi-target criteria while A4 and A5 received 10.

Method A1 had no co-eluting peaks, excellent peak shape, and good separation from its nearest neighbors. It was not selected as a “best” method because it didn’t find multiple target peaks, although visual inspection of composites show it as a good Secondary candidate to A5.

For SFC, Method A5 is ideal. For LC, Method B1 is best.

## Conclusions

- Implementation of the Separation Value criteria provides a facile way to track unknown component retention times assuring that target co-eluting peaks are not present. Additionally, the Separation value, along with other key parameters, provides a quantitative measure used to determine the “best” separation conditions.
- The algorithms utilize data from across every run of a sample as part of the knowledge base for that sample. For instance, if there are multiple targets in at least one sample, the algorithm expects to find multiple targets in every sample and will score each run accordingly (10 for multiple targets; 0 for a single target).
- Since implementation of the method scoring approach has chosen appropriate methods >95% of the time for approximately 50 samples.
- Overall throughput, from submission to purification, has been reduced by > 16 hours per sample with combination of walk-up initiation of the sample screen (the elimination of the batching approach to method screening) and the use of ASEExpress to automate data analysis.
- The utilization of Analytical Studio Professional and ASEExpress have successfully facilitated the analysis and visualization of the samples for this screening and purification workflow.