

# Automated Method Development for High Throughput Purification at Nurix Therapeutics

Shayla Masters, Robert Kettenstock, Brent Murphy, Amber Guillen

Nurix Therapeutics, San Francisco, CA, USA

## Background

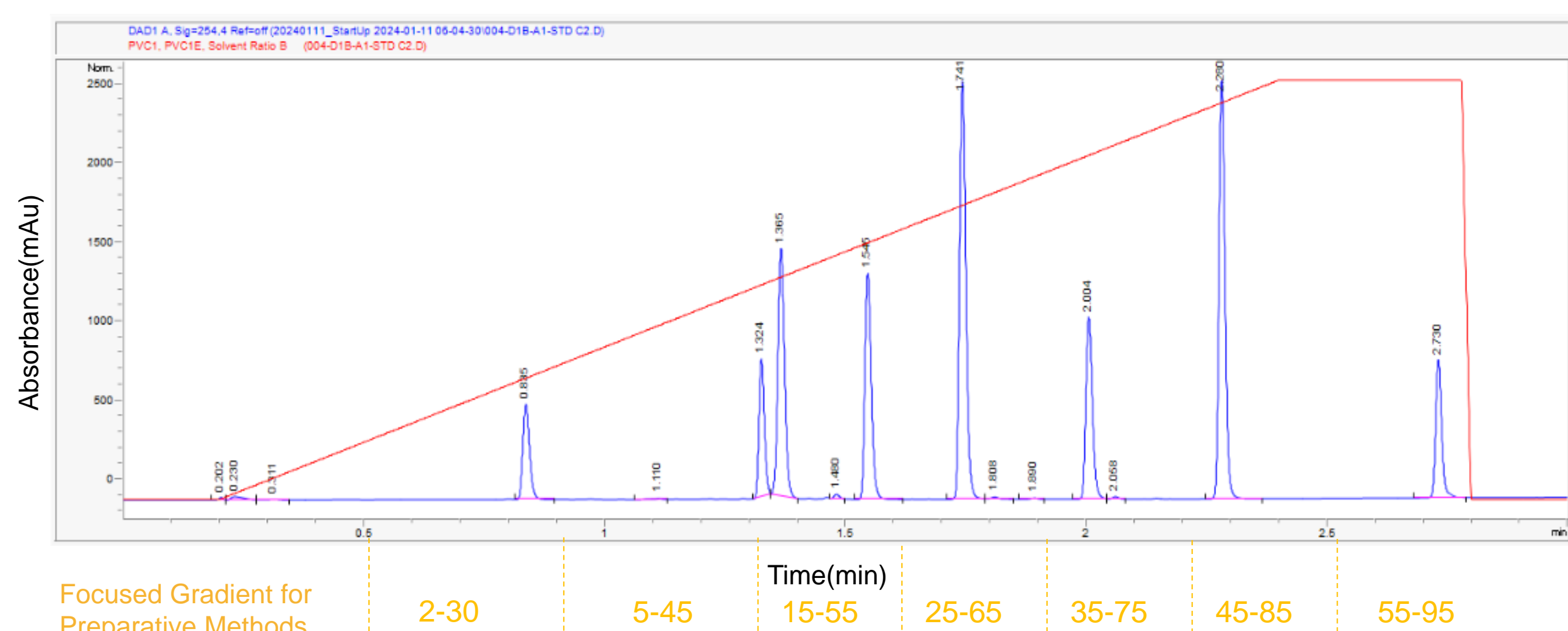
Focused gradients are commonly used in purification workflows to quickly develop preparative conditions for a large diversity of samples. To maximize efficiency, it is common to only consider single screening and preparative conditions.

At Nurix, we use Analytical Studios Pro (Virscidian, Inc.), a chromatography and mass spectrometry analysis software package, which allows analysts to interpret large data sets. We have customized this software to assist with preparative method selection factoring in multiple stationary phases, mobile phases, sample properties, and requested product specifications. This platform supports singleton and library workflows and has enabled a ten-fold increase in productivity per analyst.

## Results

Figure 1. Focused Gradients at Nurix Therapeutics

A. Three minute 5-95 %ACN screen on Agilent 1290 with PDA and SQD mass spec analytical of 8 peak standard. Red: ACN gradient, Yellow: Corresponding focused gradients on prep



B. List of compounds in standard based on elution and is used to create 7 focused gradient bins for prep.

Peak Elution on 05-95%	Compound	Focused Preparative Gradient (%B)
1	2-acetamidophenol	2-30
2	Cortisone	
3	Ethylparabens	15-55
4	Propylparabens	
5	Flavone	25-65
6	Progesterone	35-75
7	3-(4-tertbutylphenoxy)-benzaldehyde	45-85
8	2-(2H-benzotriazole-2-yl)4-(1,1,3,3-tetramethylbutyl)phenol	55-95

C. Example of a preparative method with a 10-minute focused gradient.

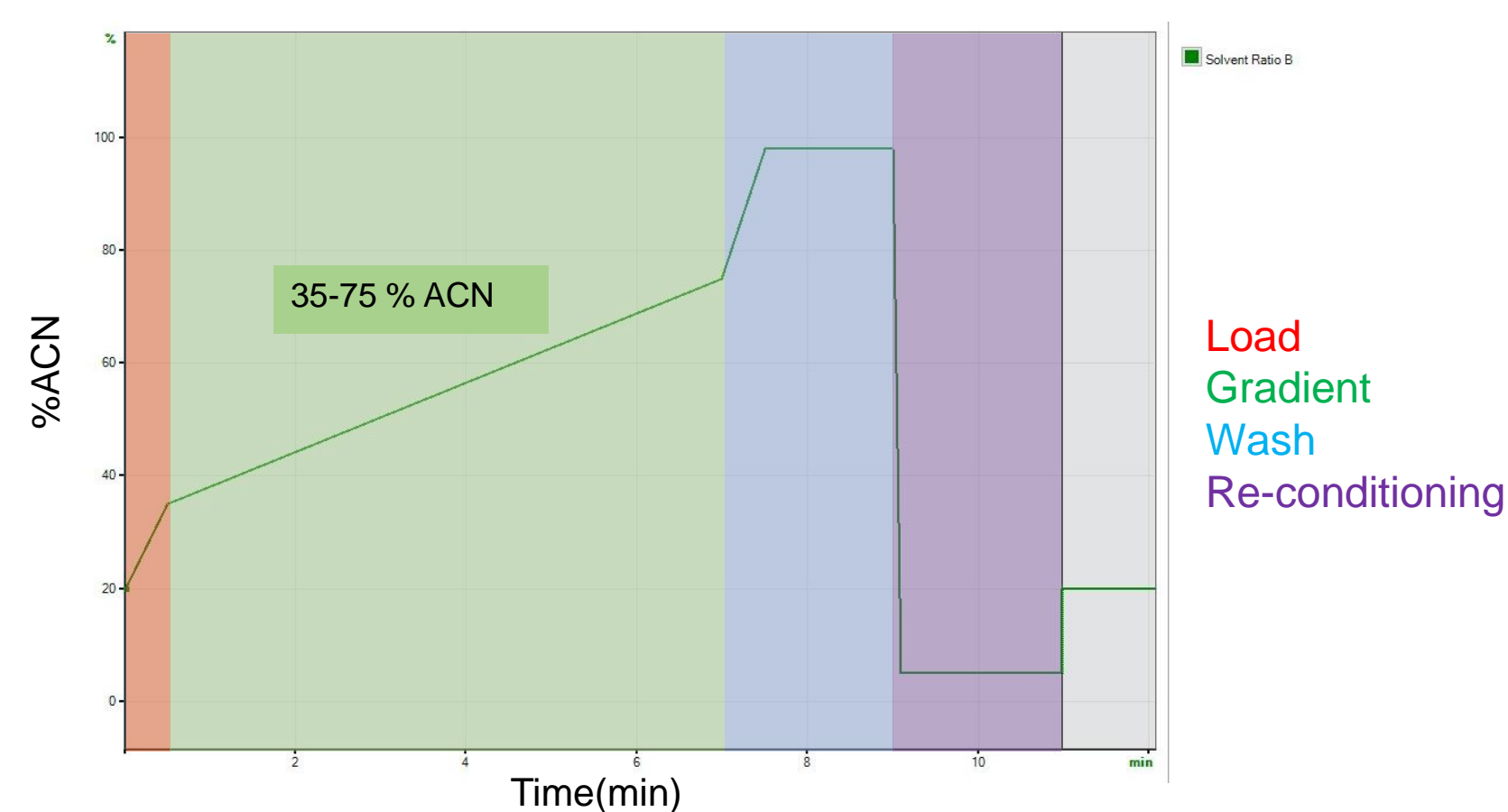
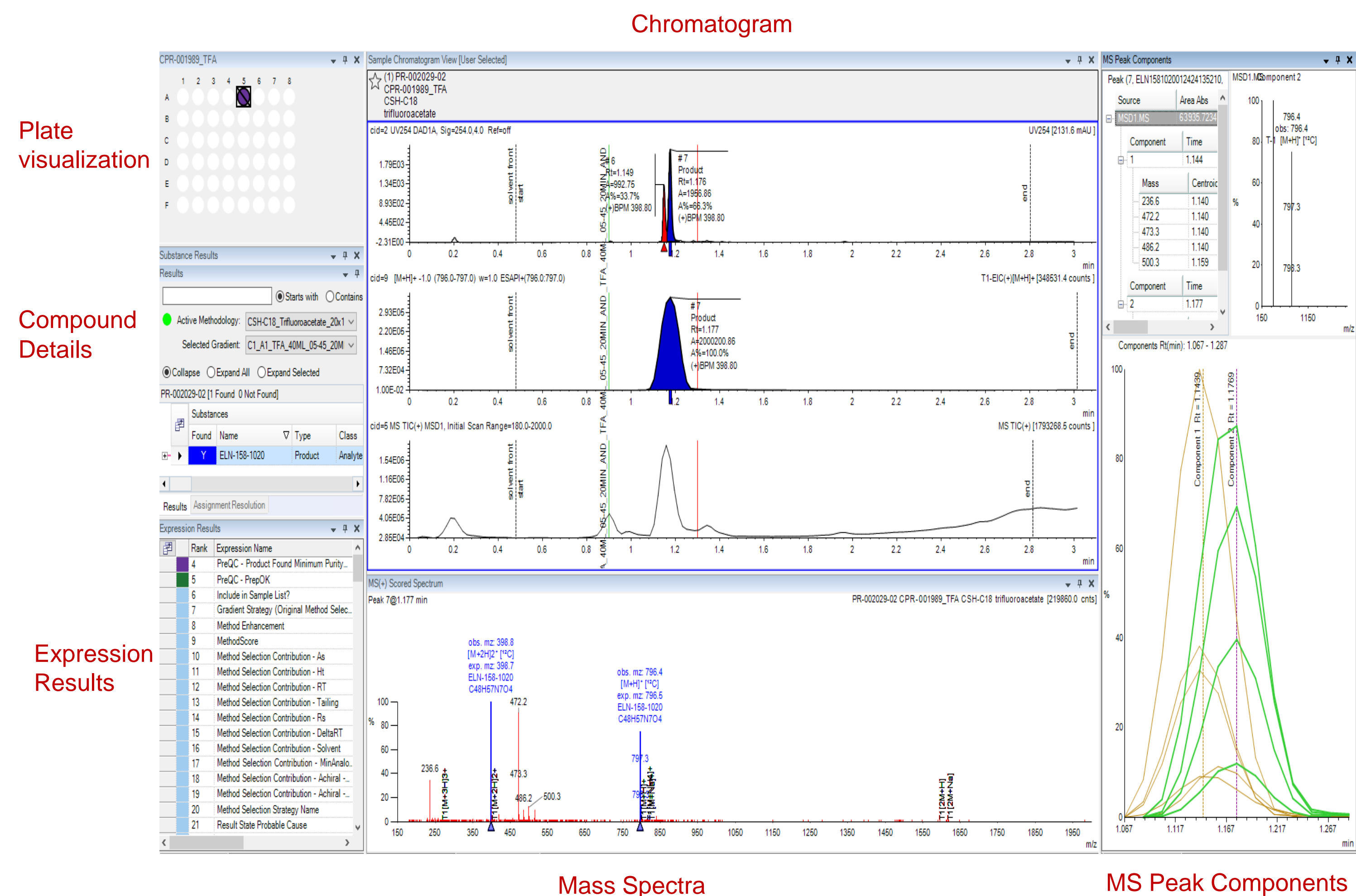


Figure 2. Data Analysis in Analytical Studio Pro

A. Visual representation of screening data taken from Virscidian's Analytical Studios Pro (ASPro). Middle: Chromatograms (UV,TIC,XIC). Top left: Visualization of the screened plate. Left middle: Compound details, such as sample ID and molecular formula and Expression results. Bottom: Mass spectra (TIC). Right: MS Peak Components - total ion chromatogram into individual ion intensity relative to time.



B. The isotopic fingerprint is used by ASPro for peak identification.

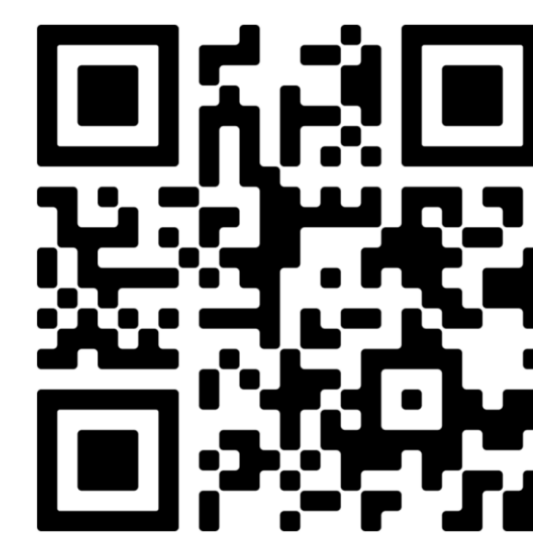
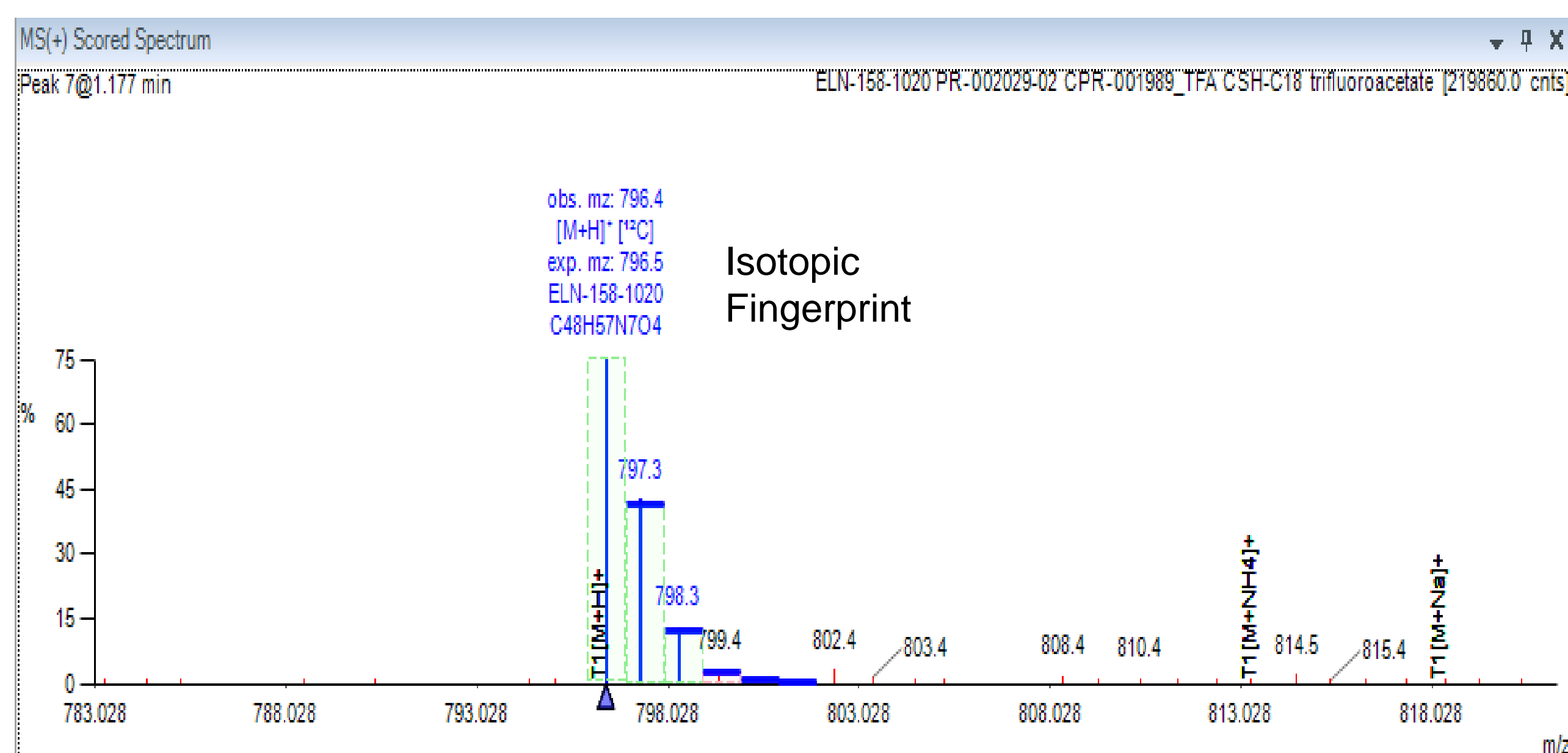


Figure 3. Decision Tree for Method Selection

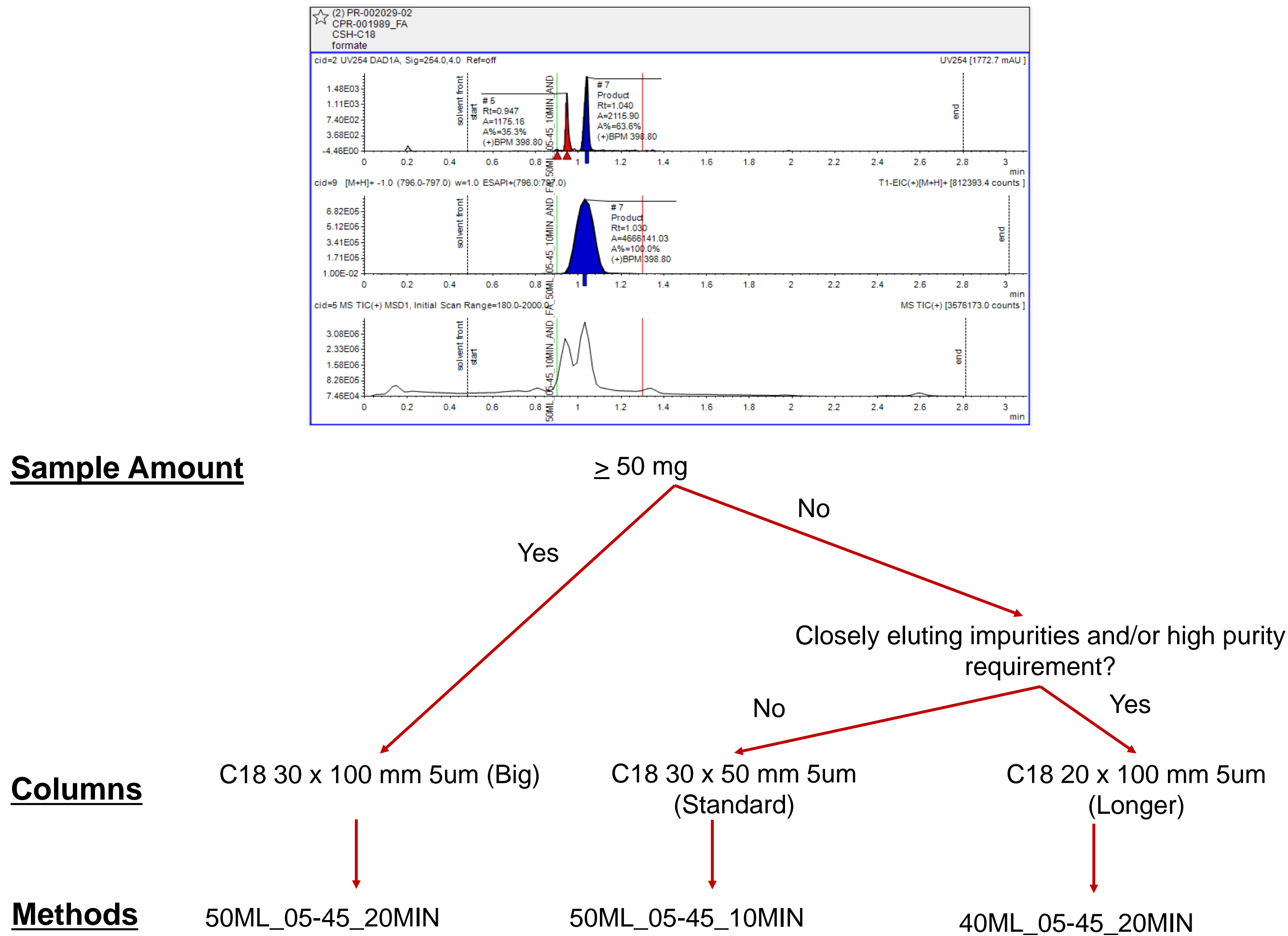


Figure 3. By looking at the reaction scale and purity requirements of each sample, Nurix uses this customized decision tree to determine preparative methodology.

Figure 4. Method Scoring

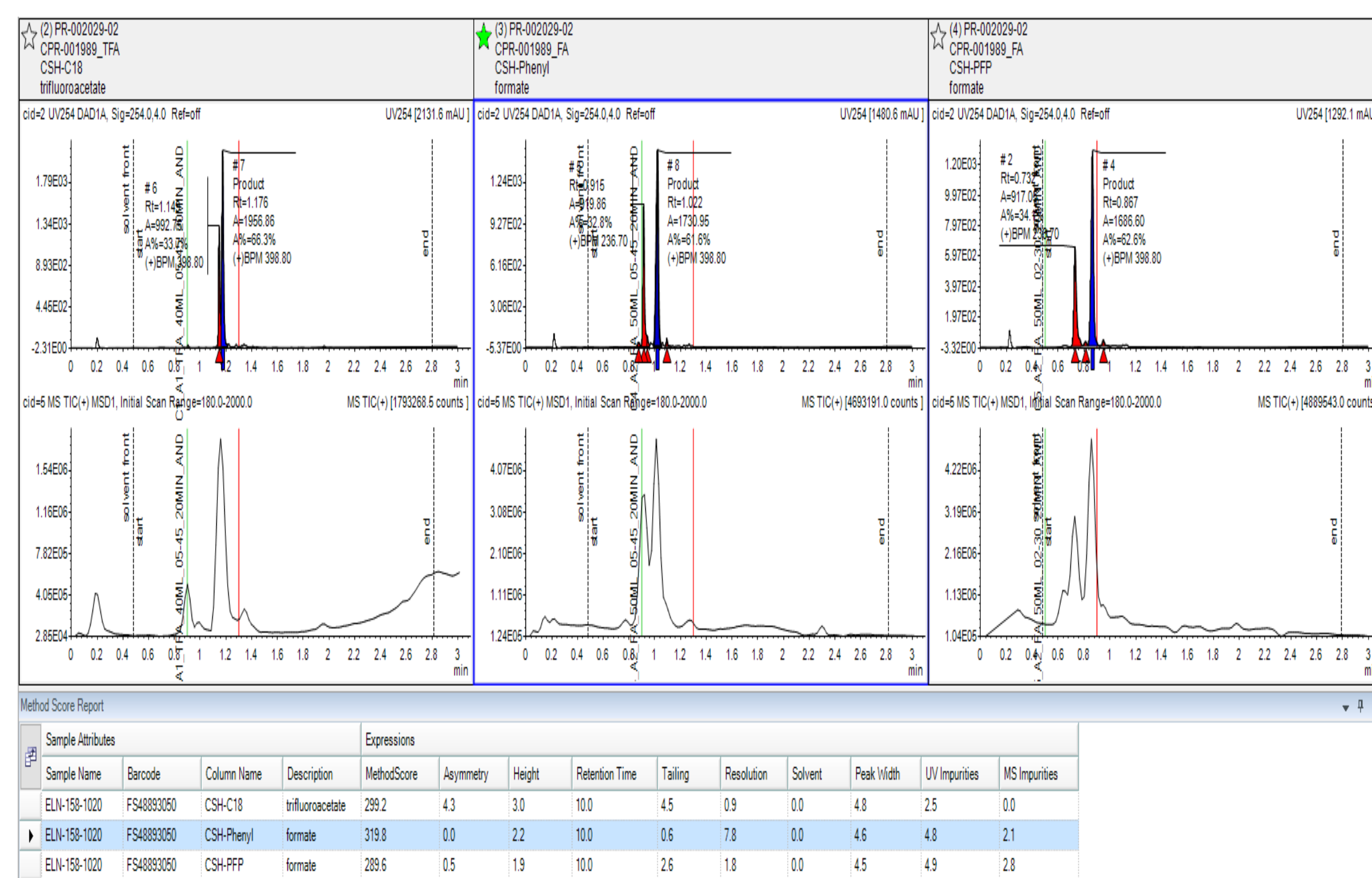


Figure 4. ASPro generates a "Method Score" for each condition based on resolution, asymmetry, impurities, UV and MS. The condition with the highest Method Score is moved forward to the prep stage.

Figure 5. Preparative Sequence Generation

A. Nurix organizes prep sequences to maximize efficiency.

LOCATION	SAMPLE NAME	TARGET MASS	INJ VOL	ENHANCED GRADIENT
D3F-B3	F548893075	809.4	600.00000	C1_A2_FA_40ML_05_EQUIV
D3F-C1	F548998460	1035.5	1250.00000	C3_A2_FA_50ML_05-45_20MIN_AND_P3
D3F-C2	F548998459	1021.5	1250.00000	C3_A2_FA_50ML_05-45_20MIN_AND_P3
D3F-C3	F548998458	995.5	1250.00000	C3_A2_FA_50ML_05-45_20MIN_AND_P3
D3F-C4	F548998457	1023.6	1250.00000	C3_A2_FA_50ML_05-45_20MIN_AND_P3
D3F-A5	F548998423	1042.4	750.00000	C2_A1_TFA_50ML_15-55_10MIN_AND_LowUV
D3F-B1	F548737569	668.3	1250.00000	C2_A2_FA_50ML_05-45_10MIN_AND_LowUV
D3F-A4	F548998955	1158.4	450.00000	C2_A2_FA_50ML_05-45_10MIN_AND
D3F-B3	F548893075	809.4	600.00000	C1_A1_TFA_40ML_05_EQUIV
D3F-B2	F548893076	781.4	600.00000	C1_A1_TFA_40ML_05-45_20MIN_AND
D3F-A6	F548998984	662.2	950.00000	C1_A1_TFA_40ML_25-65_20MIN_AND
D3F-A1	F548998949	1142.4	450.00000	C1_A1_TFA_40ML_35-75_20MIN_AND
D3F-A5	F548998423	1042.4	750.00000	C2_A1_TFA_50ML_15-55_10MIN_AND_LowUV_LowMS
D3F-A7	F548999006	662.2	1650.00000	C2_A1_TFA_50ML_35-75_10MIN_AND
D3F-A8	F548998981	662.2	950.00000	C2_A1_TFA_50ML_35-75_10MIN_AND_LowMS
D3F-A2	F548998943	1128.4	450.00000	C2_A1_TFA_50ML_35-75_10MIN_AND

B. At Nurix, we have included method enhancements to reduce the number of samples not collected on prep.

Enhancement Name	PreQC Trigger Conditions	Agilent Settings	Expression Output
High Molecular Weight	Target Mass > 1200	Charge 2	_HIMW
Low UV Response	UV Area of Best Wavelength < 100 UV threshold = 10		_LowUV
Low MS Response	MS Area of M+H < 100000	MS threshold = 5000	_LowMS
Low Solubility	Manual Assignment	Strong solvent sandwich	_P3

## Conclusions

- Manual preparative method development is labor intensive and prone to error
- Nurix uses ASPro to quickly analyze multiple screening conditions.
- Automated method selection with human review allows for fast method identification.
- Nurix creates prep sequences that switch between modifiers, columns, and include method enhancements.
- Removing majority of manual labor in preparative method selection has allowed Nurix Therapeutics to increase productivity by 10x.

## Disclosures

All authors are past or current employees of Nurix Therapeutics and hold company stock or stock options.