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アプリケーションノート

Scaling a Separation from UPLC to Purification using Focused Gradients

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Abstract

This application note will discuss the use of focused gradients to maintain selectivity and resolution and to

allow UPLC screening to be applied to preparative samples. This will offer the substantial time savings associated with UPLC to customers in the preparative environment.

Introduction

Purification laboratories face many of the same challenges that their counterparts in analytical laboratories face: the need to increase throughput and efficiency without sacrificing quality and quantity. Successful performance of a purification lab is measured in the ability to produce pure fractions in sufficient quantities in a timely manner.

UltraPerformance LC (UPLC) has been widely accepted by chromatographers because of the improvements over HPLC in sensitivity, resolution, and speed of separations. Now scientists are beginning to explore the use of this technology in the sample screening process as a screening tool to evaluate samples prior to purification.

A typical run time for analytical screening in a preparative lab is 10 minutes. By capitalizing on the efficiency of UPLC, a 10-minute run time can be shortened to as little as one minute. This offers substantial time savings enabling for greater capacity, but also fits into the “fail fast and fail cheap” motto adopted by many pharmaceutical companies.

Figure 1. The mass-directed AutoPurification System.

Experimental

A standard solution of pharmaceutical-like compounds was prepared to simulate the conditions under which many purification systems operate.

UPLC Conditions

LC system:	ACQUITY UPLC System with ACQUITY UPLC Photodiode Array (PDA) Detector
Column:	ACQUITY UPLC BEH C_{18}, 1.7 µm, 2.1 x 50 mm
Injection vol.:	2.0 µL
Flow rate:	0.8 mL/min, 2.1 x 50 mm
Mobile phase A:	0.05% Formic acid in acetonitrile
Mobile phase B:	0.05% Formic acid in water
Gradient:	Generic 5% to 95% over two minutes Focused Gradient

HPLC Conditions

LC system:	AutoPurification System
Column:	XBridge Prep OBD C_{18},5 µm, 19 x 50 mm XBridge C_{18}, 5 µm, 4.6 x 50 mm
Injection vol.:	200 µL
Mobile phase A:	0.05% Formic acid in acetonitrile
Mobile phase B:	0.05% Formic acid in water

Flow rate:	22 mL/min
Gradient:	0 to 0.25 min, 2% B to initial % B 0.25 to 1.61 min, initial % B to end % B 1.61 to 1.86 min, end % B to 95% B 1.86 to 2.71 min, 95% B 2.71 to 2.72 min, 95% B to 2% B

MS Conditions

MS system:	3100 Mass Detector
Ionization mode:	Positive
Switching time:	0.05 sec
Capillary voltage:	3 Kv
Cone voltage:	60 V
Desolvation temp.:	350 °C
Desolvation gas:	500 L/Hr
Source temp.:	300 °C
Acquisition range:	150 to 700 amu
Acquisition rate:	5000 amu/sec

Results and Discussion

In order to maintain the selectivity and resolution achieved by analytical analysis, the overall cycle time of a preparative analysis must be increased almost nine-fold.[1] This long cycle time is not practical for most

separation scientists. Therefore, we look to focused gradients to maintain selectivity and resolution in UPLC screening.

The UPLC separation of the sample shows the compound of interest eluting at 0.48 min, and is partially resolved from the peak at 0.51 min.

The separation is first directly scaled to a 19 x 50 mm XBridge Prep OBD C_{18} Column. The XBridge chemistry is built on the same second-generation bridged ethyl hybrid (BEH) particle as the ACQUITY UPLC BEH chemistry, in order to maintain the selectivity and resolution of the analytical analysis. To maintain the resolution and selectivity, the overall cycle time must be increased over nine-fold.

In a preparative environment, where the compound of interest is being isolated from the other components in the sample, retaining analytical resolution is not as important as isolating and collecting the compound of interest.[2]

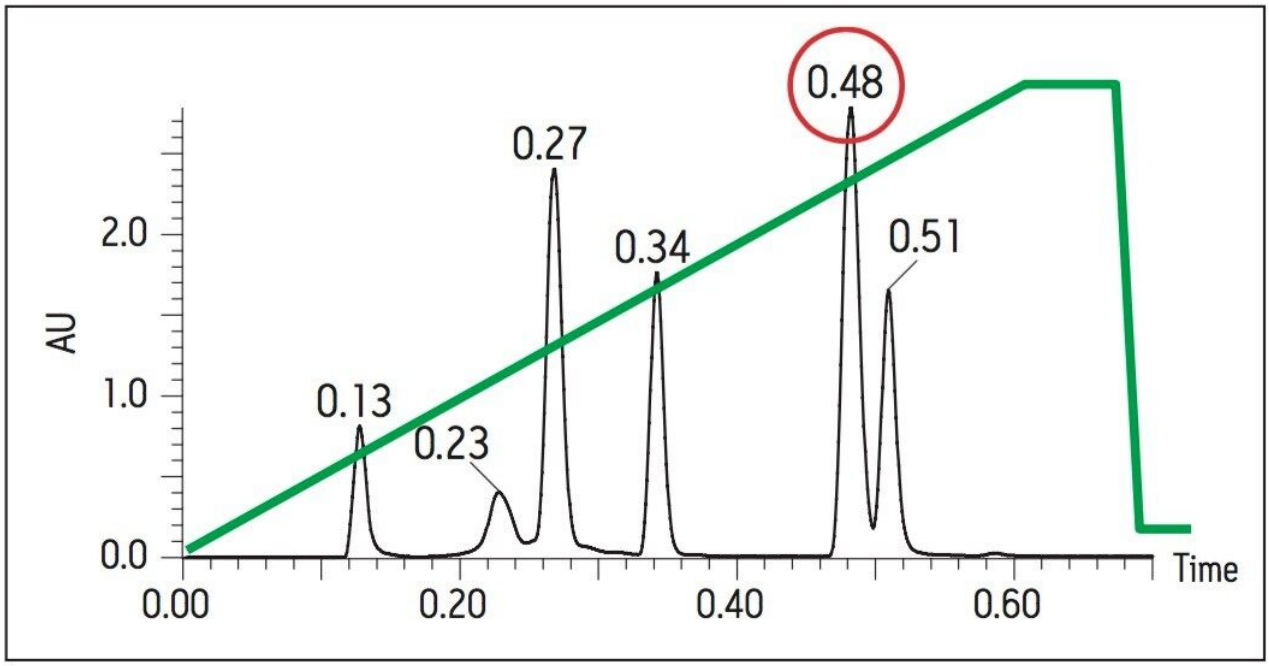

Figure 2. ACQUITY UPLC analytical separation.

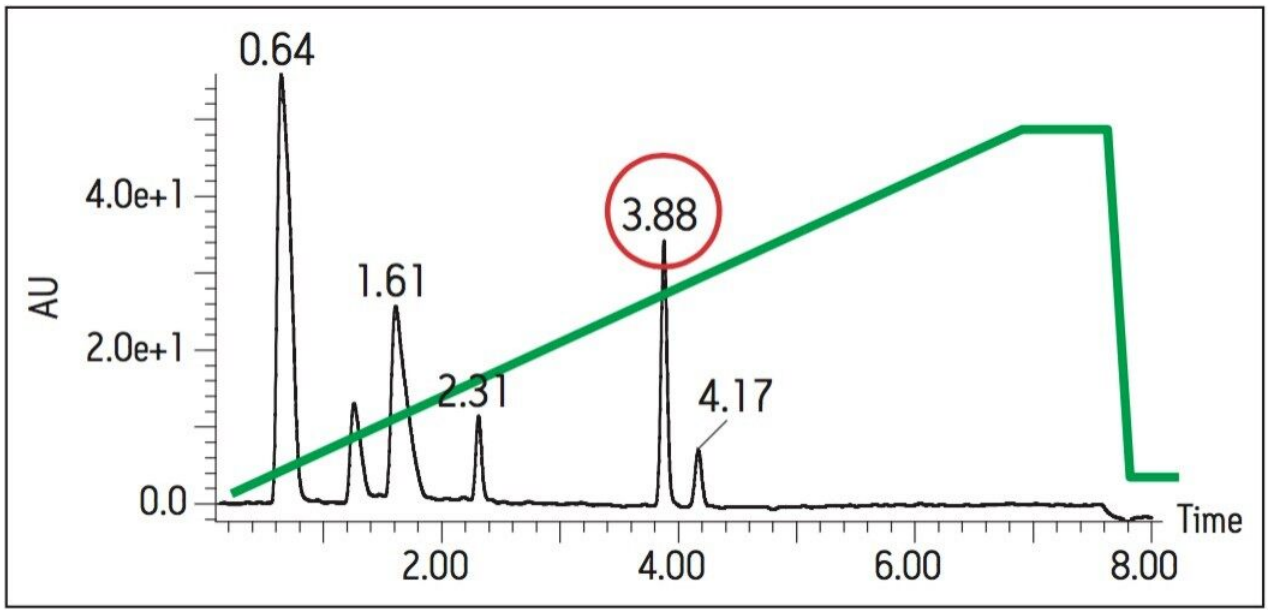

Figure 3. Direct scale-up maintains resolution and selectivity, with a run time of eight minutes.

A set of focused gradients can be created based on the relationship between percent composition and retention time. The system d-well time is used to determine that relationship.[3]

Here, in the analytical screen the mobile phase is 2% organic solvent at 0.17 minutes and 17.5% at 0.295 minutes, and so a series of gradients can be created.

The theory behind the focused gradients is the same for HPLC and for UPLC, but the time window for the UPLC gradient is much smaller.

Based on Table 1, method C is selected to isolate the compound that eluted at 0.48 min in the UPLC analysis. Using the focused gradient, the separation and isolation of the compound was carried out in three minutes.

Method	Time (min)	Time (min)	% B start	% B end
A	0.17	0.295	2	17.5
B	0.295	0.42	17.5	33
C	0.42	0.545	33	48.5
D	0.545	0.67	48.5	64
E	0.67	0.795	64	79.5
F	0.795	0.92	79.5	95

Table 1. UPLC retention time windows and corresponding focused preparative gradient composition.

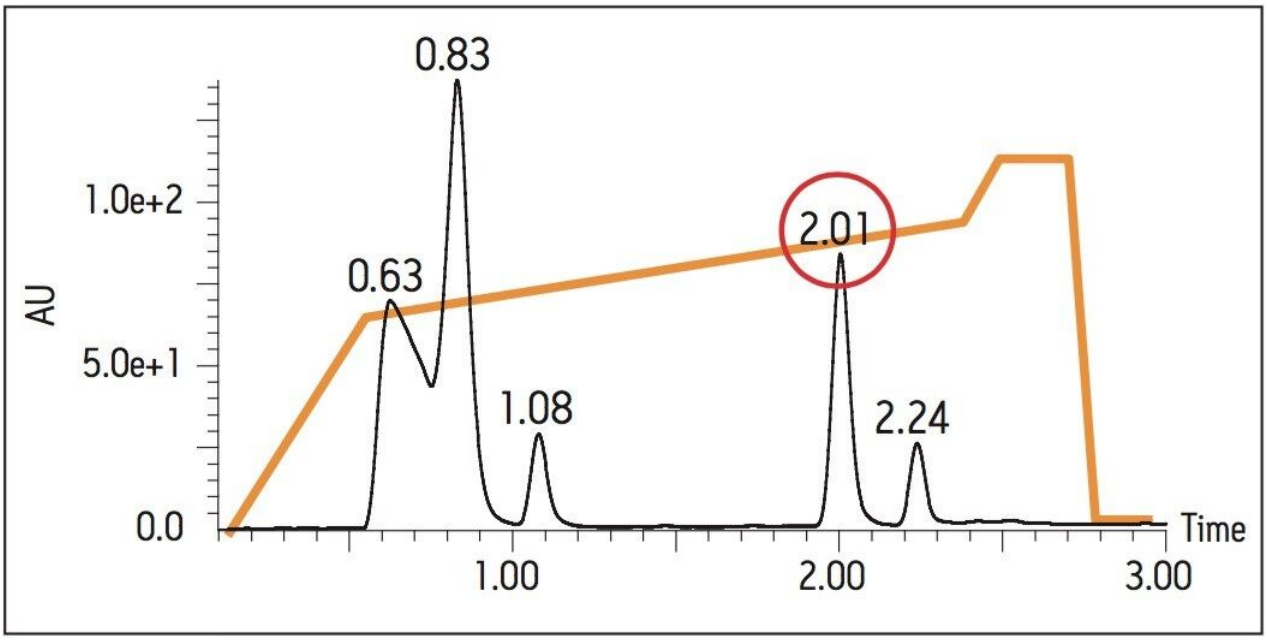

Figure 4. Separation of the compound of interest using a three-minute focused gradient.

UPLC Library Purity Screening

This same methodology can be applied to the purity screening and purification of a large sample library. The ACQUITY UPLC System’ s large capacity (22 384-well plates) and the rapid analysis cycle time provide the ideal tool for high throughput library screening. Data is processed and handled using AutoPurify, part of

the FractionLynx Application Manager.[4]

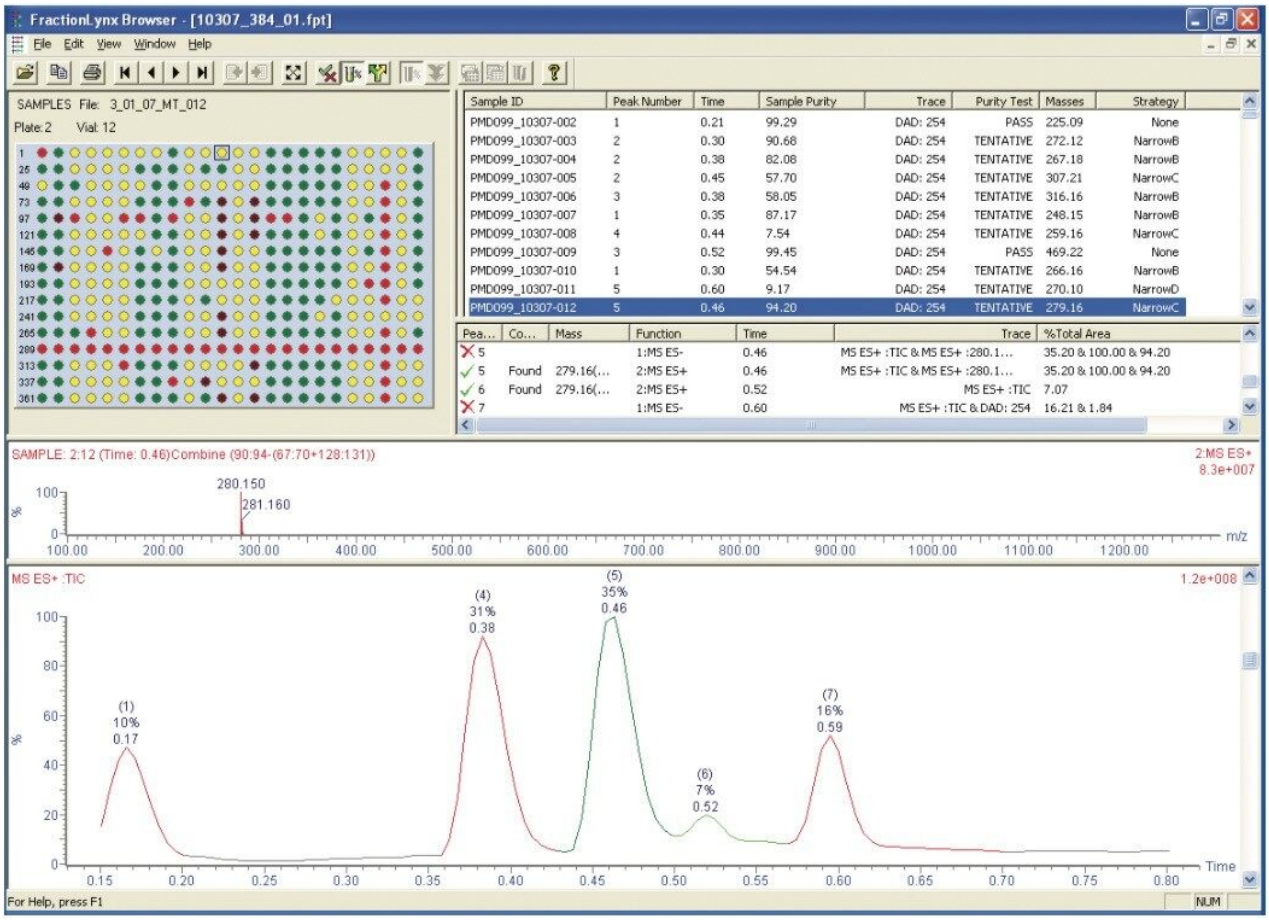

Figure 5. AutoPurify processing report showing the color coded purity and found/not found of a 348-well plate.

Focused Library Purification

AutoPurify automatically selects the samples requiring purification and the corresponding focused preparative method.

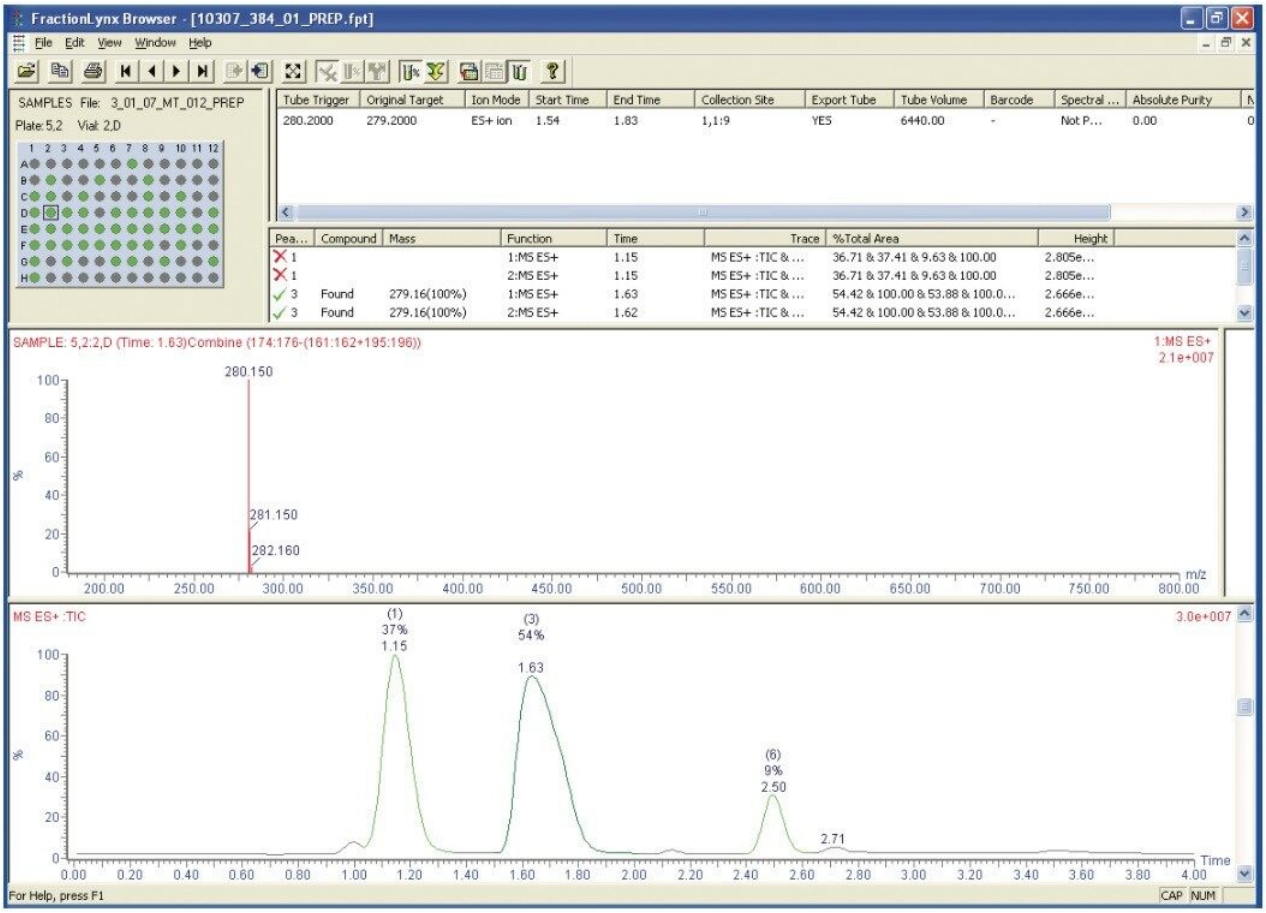

Figure 6. AutoPurify processing of the UPLC screening library.

UPLC Fraction Analysis

The substantial time savings associated with analytical screening can be magnified by incorporating UPLC into the analysis of the collected fractions. The collected fractions are analyzed to determine the new sample purity, and sample lists are automatically generated for each step of the process. By incorporating fraction analysis by UPLC into the workflow, the efficiency of the lab is further increased.

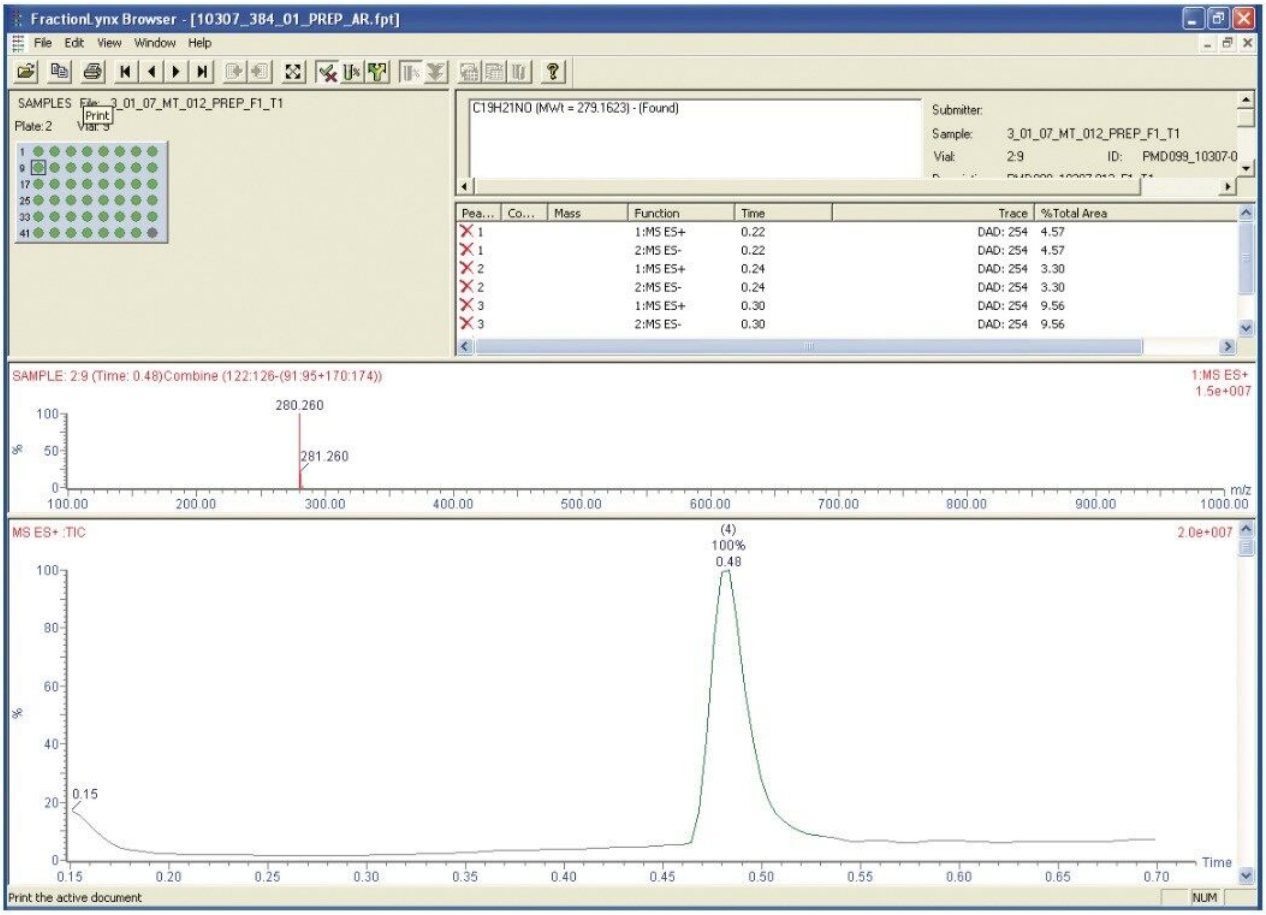

Figure 7. AutoPurify processing of the UPLC analysis of the collected fractions.

Conclusion

- Scale-up from UPLC to preparative HPLC in an efficient manner is possible with the use of focused gradients.
- The efficiency of UPLC can be carried through to purification, offering a substantial increase in throughput and productivity.
- The AutoPurify capabilities of FractionLynx allows for automation from the initial UPLC QC, through purification, to UPLC fraction analysis.
- AutoPurify is also capable of automatically selecting a focused preparative gradient based on the analytical results, giving better quality purification and eliminating the need for expert manual invention.

References

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2. Cleary R, Lefebvre P. The Impact of Focused Gradients on the Purification Process, Waters Application Note. 2007; 720002284EN <https://www.waters.com/nextgen/us/en/library/application-notes/2007/the-impact-of-focused-gradients-on-the-purification-process.html> .
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4. Cleary R, Lefebvre P. Purification Workflow Management, Waters Application Note. 2006; 720001466EN < https://www.waters.com/nextgen/us/en/library/application-notes/2007/purification-workflow-management.html> .

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