DEVELOPMENT OF PRACTICAL RULES FOR HIGH-THROUGHPUT MS-DIRECTED PURIFICATION OF A MEDICINAL CHEMISTRY LIBRARY BASED ON UPLC/MS

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INTRODUCTION

Purification laboratories face many of the same challenges that their counterparts in analytical laboratories face, increase throughput and improve performance. 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 purification process as a screening tool to evaluate samples prior to purification.

In order to maintain the selectivity and resolution achieved by the analytical analysis, the overall cycle time of a preparative analysis must be increase almost 9 fold.[1] This long cycle time is not very practical for most separation scientists.

This poster will discuss the use of focused gradients to maintain selectivity and resolution and 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.

Figure 1. ACQUITY UPLC® MS/PDA/ELSD System.

Figure 2. Waters® Mass-Directed AutoPurification™ System.

METHODS

UPLC conditions

LC system:	ACQUITY UPLC system with ACQUITY UPLC PDA
Column:	ACQUITY UPLC BEH C18, 2.1 X 50 mm, 1.7um
Injection volume:	2.0µl
Flow rate:	0.8 ml/min 2.1 x 50 mm
Mobile phase:	A = Acetonitrile/ 0.05% Formic Acid B = Water/ 0.05% Formic Acid
Gradient:	Generic 5 to 95% over 0.7 minutes

HPLC conditions

LC system:	Waters AutoPurification system	
Columns:	Waters XBridge™ Prep OBD™ C18 5um 19 X 50 mm	
Injection volume:	200 µl	
Mobile phase:	A = Acetonitrile/ 0.05% Formic Acid B = Water/ 0.05% Formic Acid	
Flow rate:	22 ml/min	
Gradient:	0 – 0.25 min	2 – Initial %B
	0.25 – 1.61	Initial – End %B
	1.61 – 1.86	End – 95 %B
	1.86 – 2.71	95 %B
	2.71 – 2.72	95 – 2 %B

MS conditions

MS system:	Waters 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 – 700 amu
Acquisition rate:	5000 amu/sec

Solutions of pharmaceutical like compounds were prepared to simulate the conditions under which many purification systems operate.

SCALING UP FROM UPLC

UPLC Separation

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

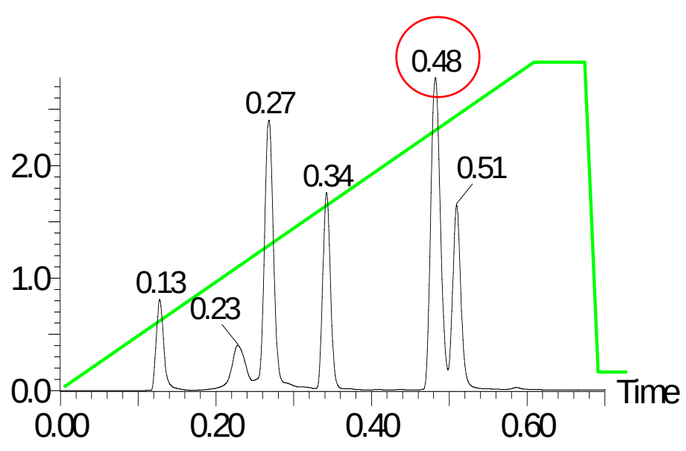

Figure 3. ACQUITY UPLC separation

Scaled Generic Preparative Separation

The separation is first directly scaled to a 19 X 50mm XBridge™ Prep OBD™ C18 column. The XBridge™ chemistry is built on the same second generation Bridged Ethyl Hybrid (BEH) particle as the ACQUITY UPLC BEH Technology™, in order to maintain the selectivity and resolution of the analytical analysis. In order to maintain the resolution and selectivity, the overall cycle time must be increased over 9 fold.

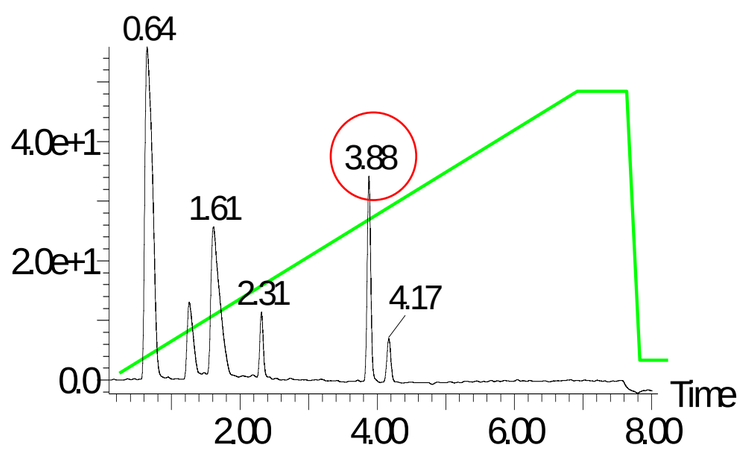

Figure 4. Direct scale-up maintains resolution and selectivity, with a run time of 8 minutes

Applying Focused Preparative Gradients

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].

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

Method	Time	Time	% 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

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

Focused Preparative Separation

Based on Table 1, method C is selected to isolate the compound that eluted at 0.48 minutes in the UPLC analysis. Using the focused gradient, the separation and isolation of the compound was carried out in 3 minutes.

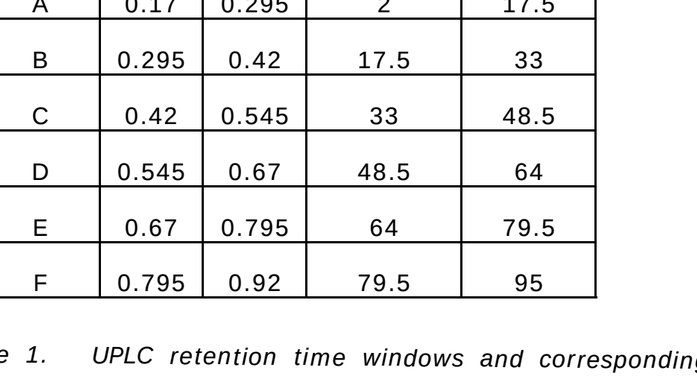

Figure 5. Separation of the compound of interest using a 3 minute focused gradient

LIBRARY PURIFICATION

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 throughput library screening. Data is processed and handled using AutoPurify™.[4]

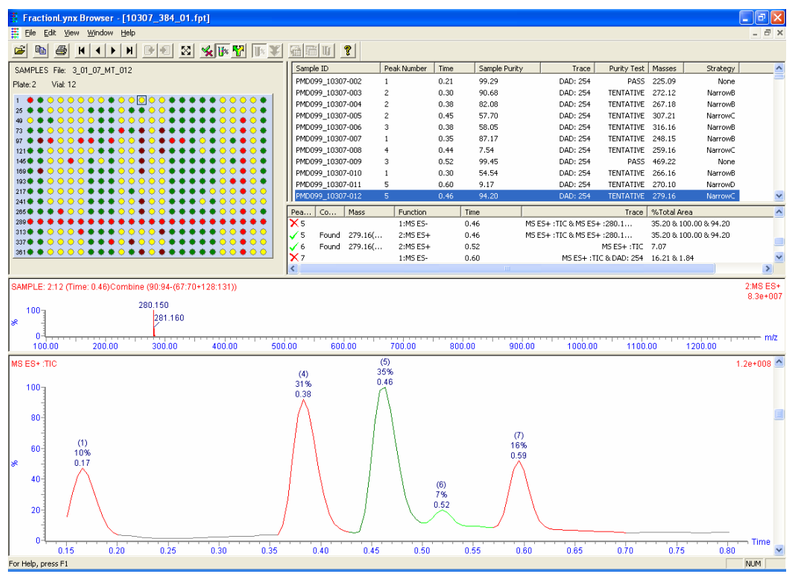

Figure 6. AutoPurify processing report showing the color coded purity and found / not found of a 348-well plate.

Focused Library Purification

AutoPurify automatically selects the samples requiring purification and the correspond preparative method. All methods are directly scaled from the UPLC separation.

Figure 7. AutoPurify processing of the UPLC screening library

UPLC Fraction Analysis

The collected fractions can be analyzed to determine the new sample purity. Sample lists is automatically generated for each step of the process.

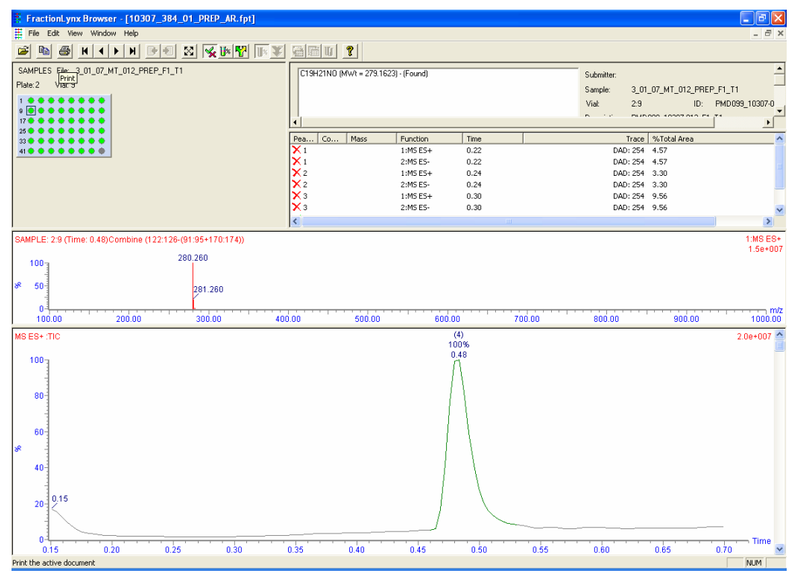

Figure 8. AutoPurify processing of the UPLC analysis of the collect fractions

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 the purification, to UPLC fraction analysis.**
- **The AutoPurify software is also capable of automatically selecting a narrow focused preparative gradient based on the analytical results, giving greater quality purification and eliminating the need for expert manual invention to handle the "one-offs".**

REFERENCES

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4. Purification Workflow Management, Cleary and Lefebvre May 2006, document # 720001466EN

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