

# A COMPARISON BETWEEN HPLC AND UPLC FOR METABOLITE SCREENING IN DRUG DISCOVERY USING TOF-MS

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## INTRODUCTION

LC-MS has revolutionised drug metabolism applications with new technologies such as triple quadrupoles, ion traps and TOF instrumentation. In drug metabolism there is a requirement to obtain answers quickly and HTS plays an important role here. Faster chromatographic times are necessary to keep up with the number of samples to be analysed and also to cut down the time taken to report the experimental findings. With all of these in mind, running samples faster may also have some implications associated with these such as ion suppression in the MS system since the biological matrices used are very complex. Not having an adequate separation strategy may ultimately impact the output in the MS system. Improving the speed of analysis without sacrificing the chromatographic resolution will allow us to obtain superior separations with high peak capacity, thus reducing co-elution of metabolites and also enhance the sensitivity in the MS system but reducing ion suppression.

The heart of UPLC<sup>TM</sup> is in the use of sub 2  $\mu$ m particle sizes and the ability to sustain elevated pressures in the order of 10,000 -12,000 psi. To investigate this we have employed an Ultra Performance Liquid Chromatography system (UPLC<sup>TM</sup>) (Figure 1) and an HPLC system to compare the difference between these two technologies when coupled with a TOF-MS and triple quadrupole mass spectrometer for metabolism studies. In order to show the potential of this particular approach, we will be showing a number of analytical runs in which we compare different in vitro incubations for Buspirone and Midazolam.

## METHODS

### MS Methodology

Mass Spectrometer: Q-ToF Premier<sup>TM</sup> and Quattro Premier  
MS scan range: 70-900 Da  
Mode of Operation: + ion mode ESI

### UPLC<sup>TM</sup> Methodology

Waters ACQUITY UPLC<sup>TM</sup> System  
Acquity BEH C<sub>18</sub> Column 50x2.1mm id, 1.7 $\mu$ m, 40°C  
Mobile phase A: 0.1 % formic acid  
Mobile phase B: acetonitrile  
Flow rate: 0.8 mL/min  
Gradient: 0-1.75 min 5%-70% B, 1.75-1.9 min 100% B  
Injection volume: 5  $\mu$ L

### HPLC Methodology

#### Waters Alliance HT 2795 (HPLC) System

Waters Xbridge BEH C<sub>18</sub> Column 50x2.1mm id, 3.5 $\mu$ m, 40°C  
Mobile phase A: 0.1 % formic acid  
Mobile phase B: acetonitrile  
Flow rate: 1) 0.8 mL/min;  
2) 0.6 mL/min;  
3) 0.3 mL/min.  
Gradient: 1) 0-1.75 min 5%-70% B, 1.75-1.9 min 100% B;  
2) 0-2.33 min 5%-70% B, 2.33-2.53 min 100% B;  
3) 0-4.67 min, 5%-70% B, 4.67-5.33 min, 100% B.  
Injection Volume: 5  $\mu$ L

### Samples

Rat microsomal incubations for Midazolam and Buspirone was carried out at 100 mM and diluted prior to injection 1/1 with water +0.1% formic acid. With respect the analysis of Verapamil in rat urine the sample was diluted 1/10 with water + 0.1% formic acid and injected directly into the LC/MS System

## RESULTS

- Figure 1 shows the results of the same sample analyzed by UPLC and HPLC (3 different flow rates also compared).
- With UPLC a faster run time under 1 minute was achieved without any loss in chromatographic resolution.

Microsome Incubation of Buspirone  
Selected Ion Chromatograms of Hydroxy-Buspirone (*m/z* 402)

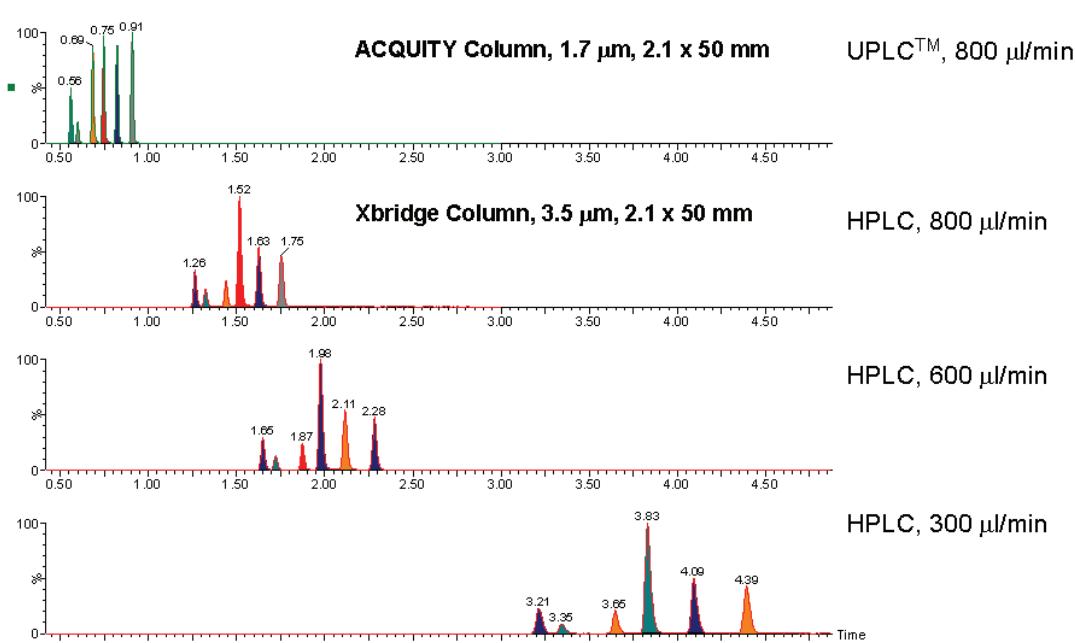


Figure 1. Comparison of UPLC vs. HPLC (at different flow rates) for the detection and separation of the hydroxylated metabolites of Buspirone.

- In the case of TOF-MS instrumentation when coupled to UPLC it can be used in high resolution mode with exact mass. In this example a narrow extracted ion chromatogram window is used of 30mDa which will remove the endogenous peaks only leaving drug-related metabolites present.
- With UPLC it can be observed that 4 hydroxylated metabolites of Midazolam are detected vs 3 hydroxylated metabolites of Midazolam (Figure 2)

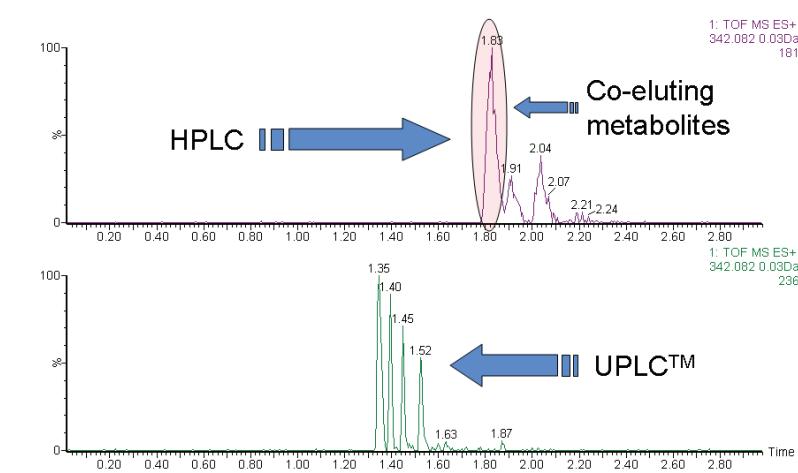


Figure 2. Comparison of UPLC vs. HPLC for the hydroxylated metabolites of Midazolam in vitro.

- In the case of in-vivo samples, UPLC also provides a much higher degree of separation when compared with HPLC as it can be seen in Figure 3.
- 4 Glucuronidated metabolites of Verapamil are detected by UPLC instead of just 2 glucuronidated metabolites by HPLC. With higher chromatographic resolution it will allow us to obtain better qualitative MS/MS data for each putative metabolite of interest

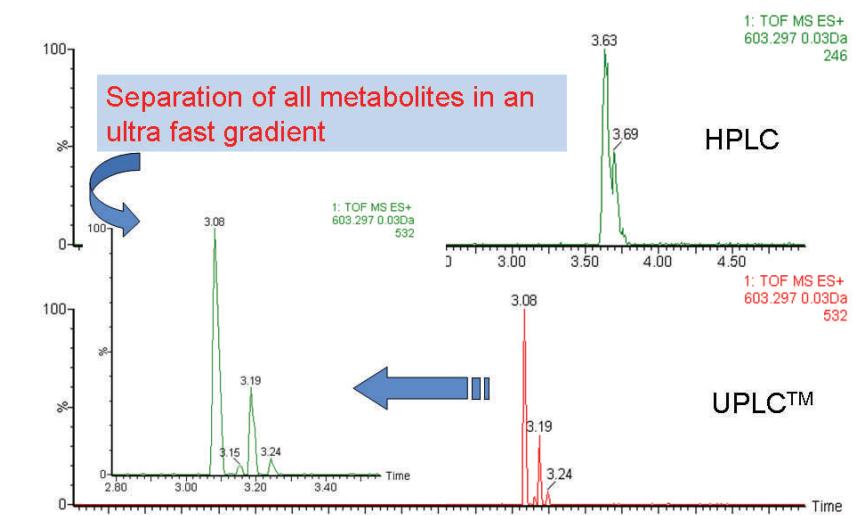


Figure 3. Comparison of UPLC vs. HPLC for the glucuronidated metabolites of Verapamil in vivo.

## CONCLUSION

- UPLC allows more information, more samples per unit time resulting in much higher productivity**
- HPLC methods are easily transferred to UPLC since the same chromatographic principles apply**