

Robert S. Plumb¹, Jose Castro-Perez², Kelly A. Johnson¹, and Michael D. Jones¹
¹Waters Corporation, Milford, MA, USA, ²Waters Corporation, Manchester, UK

INTRODUCTION

The rapid profiling of drug metabolites of candidate pharmaceuticals is an essential part of the drug discovery process, and is usually achieved by LC/MS analysis. In this task, there is a likely trade-off between throughput and the comprehensive analysis of the sample. While sample throughput can be increased, it is usually at the expense of chromatographic resolution, thus resulting in the failure to detect all of the metabolites. An alternative to traditional HPLC are high throughput chromatographic techniques such as monolithic chromatography or Ultra Performance LC™ (UPLC™). Both techniques promise higher throughput with no significant loss of resolution. To demonstrate and compare the attributes of each, we evaluate the resolution and sensitivity for the analysis of the in-vitro metabolites of verapamil, a calcium antagonist.

EXPERIMENTAL

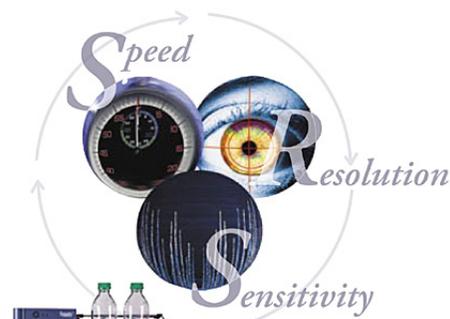
A 50 µmol solution of verapamil was incubated with rat liver microsomes for 30 minutes at 37 °C. The reaction was stopped by the addition of 2 volumes of cold acetonitrile. The sample was then centrifuged and the resulting supernatant layer was removed for analysis by LC/MS. Prior to analysis, the sample was diluted 1:5 with distilled water.

LC Conditions

LC System:	Waters® ACQUITY UPLC™ System
Column:	Chromolith™ SpeedROD RP-18e HPLC Column (Merck), 4.6 x 50 mm (monolith analysis) ACQUITY UPLC BEH C ₁₈ Column, 1.7 µm, 2.1 x 50 mm (UPLC analysis)
Mobile Phase A:	0.1% aqueous formic acid
Mobile Phase B:	Acetonitrile with 0.1% formic acid
Flow Rate:	2 mL/min. split 100 µL/min. to MS (monolith analysis) 500 µL/min., no split (UPLC analysis)
Gradient:	15–90 %B over 10 min.
Injection Volume:	10 µL (monolith analysis) 2 µL (UPLC analysis)
Sample Temp:	10 °C
Column Temp:	40 °C

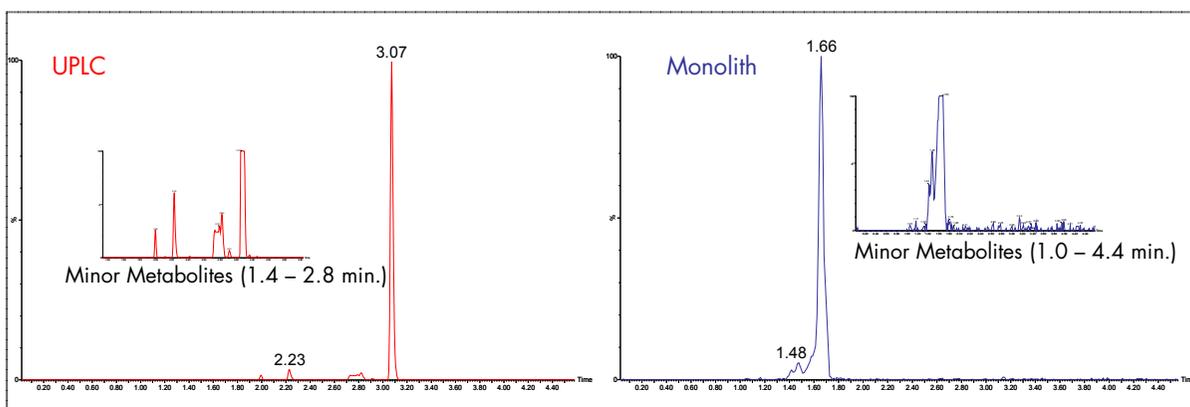
MS Conditions

MS System:	Waters Micromass® Q-ToF micro™ Mass Spectrometer
Ionization Mode:	ESI ⁺
Source Gas:	300 L/hr. at 250 °C
Acquisition Mass Range:	100–800 m/z
Cone Voltage:	35 V
Collision Energy:	10 eV
Dwell:	0.1 s
Collision Gas:	Argon



RESULTS AND DISCUSSION

The verapamil molecule can undergo metabolic dealkylation at seven different sites, resulting in seven different isobaric metabolites of verapamil, $m/z = 441.2753$. The data shown in the figure below represents the extracted ion chromatograms ($m/z = 441.2753$) from the monolithic analysis (right) and the UPLC analysis (left). As can be seen from this data, the UPLC analysis produces one major and four minor (inset, left) distinct dealkylated metabolites of verapamil, whereas the monolithic analysis results in the minor dealkylated metabolites eluting as one unresolved peak (inset, right), just before the major metabolite peak.



UPLC (left) vs. monolithic (right) analysis of the *in vitro* metabolites of verapamil

CONCLUSION

The ACQUITY UPLC System provides excellent resolution and throughput, allowing all of the major and minor metabolites of verapamil to be resolved and detected in just one short ten-minute separation. The extra resolution and sensitivity afforded by ACQUITY UPLC makes it the ideal choice for such complex mixture analysis over monolithic HPLC.

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com

Waters

For Complete Confidence

Waters, Ultra Performance LC, Micromass, Q-ToF micro, and UPLC are trademarks of Waters Corporation.
All other trademarks are property of their respective owners.
©2004 Waters Corporation Produced in the U.S.A.
Nov. 2004 720001064EN KJ-PDF

