

# Improving Detection Limits for Metabolite Identification Using StepWave Technology with SYNAPT G2-S

## GOAL

To demonstrate a new level in sensitivity and accuracy for *in vitro* microsomal incubation studies, demonstrating the use of UPLC<sup>®</sup>/MS<sup>E</sup> methodology with Waters<sup>®</sup> SYNAPT<sup>®</sup> G2-S to perform metabolite identification studies at previously unreachable concentration levels.

## BACKGROUND

Historically, metabolite identification has relied on high concentrations of substrate to provide quality data sets. Although high concentrations improve the analyst's ability to characterize data, changes in metabolic pathways and enzyme kinetics can occur, and the experiments are often not physiologically relevant when conducted at levels that may be saturating key enzymatic pathways. Additionally, many labs are decreasing incubation volumes through the implementation of automated plate liquid handling systems and miniaturized assays that are designed to handle small volumes of material to increase lab throughput, productivity, and realize cost benefits. The ability to conduct studies at lower concentrations and with less material demands parallel improvements in analytical detection technologies. Here we will demonstrate the capabilities of the ACQUITY UPLC<sup>®</sup> System coupled with a SYNAPT G2-S to enable confident metabolite identification analysis at extremely low concentrations levels.

With UPLC/MS<sup>E</sup> and SYNAPT G2-S, metabolites can be routinely detected and characterized at nM levels, providing more information at physiologically-relevant concentrations.

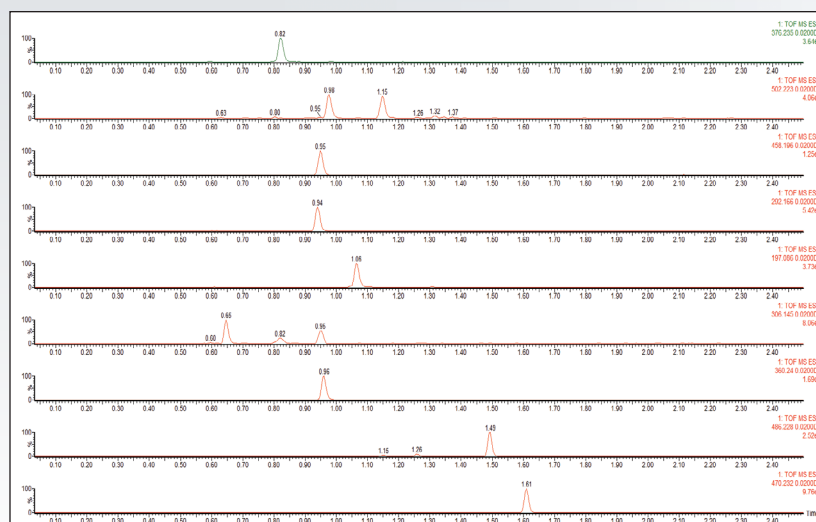


Figure 1. Extracted ion chromatograms of metabolites at 0.01  $\mu\text{M}$ .

Metabolite Name	% Area*	RT	m/z*	theor	mDa*	Detection Level (Incubation dilution, $\mu\text{M}$ )				
						10	1	0.1	0.01	0.001
Parent	60.5	1.60	470.2318	470.2323	-0.5	x	x	x	x	x
Hydroxylation	15.1	1.46	486.2276	486.2272	0.4	x	x	x	x	x
Parent-C6H3Cl	11.5	0.96	360.2405	360.2399	0.6	x	x	x	x	x
Hydroxylation-C10H11N2Cl	3.5	0.94	292.1670	292.1661	0.9	x	x	x	x	x
Parent-C15H19N3O2	2.7	1.06	197.0857	197.0845	1.2	x	x	x	x	x
Hydroxylation-C6H3Cl	2.6	0.82	376.2352	376.2348	0.4	x	x	x	x	x
Hydroxylation	1.3	1.26	486.2277	486.2272	0.5	x	x	x	x	x
Hydroxylation	1.0	1.15	486.2281	486.2272	0.9	x	x	x	x	x
Hydroxylation + Deethylation	0.8	0.95	458.1959	458.1959	0.0	x	x	x	x	x
2xHydroxylation+Desaturation-C10H11N2Cl	0.5	0.65	306.1462	306.1454	0.8	x	x	x	x	x
2xHydroxylation	0.3	0.98	502.2238	502.2221	1.7	x	x	x	x	x
2xHydroxylation	0.3	1.15	502.2227	502.2221	0.6	x	x	x	x	x

Table 1. Area and mass accuracy values shown from 0.1  $\mu\text{M}$  dilution series.

## THE SOLUTION

Human liver microsomes spiked with 10  $\mu\text{M}$  Nefazodone were incubated for 0 and 60 min at 37 °C. The samples were quenched with one volume of cold acetonitrile + 0.1% formic acid, centrifuged, and diluted with 1:1 acetonitrile:water to provide a dilution series. The samples then were analyzed using a SYNAPT G2-S coupled with an ACQUITY UPLC® System. Data acquisition was performed in positive ion, sensitivity mode. 5  $\mu\text{L}$  of sample were injected onto an ACQUITY UPLC BEH, 1.7  $\mu\text{m}$ , 2.1 x 50 mm Column and run with a 2 min gradient using a flow rate of 0.7 mL/min. The mobile phase consisted of 0.1% ammonium hydroxide (A) and methanol (B). Data were processed and analyzed using MetaboLynx™ XS v.2.0 Software.

Tabulated results for human microsomal met ID are shown in Table 1. The top 10 metabolites (top 11 actually shown) were reported for 10  $\mu\text{M}$  Nefazodone incubation and verified to be present (or absent) in four successive dilutions down to 0.001  $\mu\text{M}$  (1 nM). These top 10 metabolites were detected down to 0.01  $\mu\text{M}$  (10 nM) dilution. At the final dilution (1 nM), remaining parent and two major metabolites – all peaks > 5% total area – were still observed. Figure 1 shows the extracted ion chromatograms obtained for the metabolites in the 0.01  $\mu\text{M}$  dilution sample. Figure 2 shows the quality of MS<sup>E</sup> fragmentation data obtained for the hydroxy metabolite (1.46 min) at successive dilutions down to the 0.01  $\mu\text{M}$  dilution. Fragmentation data is clear and comprehensive down to 0.01  $\mu\text{M}$  incubation levels, and structural assignments can be confidently made even at the lowest level shown.

## SUMMARY

The StepWave™ Technology employed in the SYNAPT G2-S allows scientists to routinely and rapidly access information at levels that have been out of reach until now.

## MS<sup>E</sup> Fragment Ion Spectrum

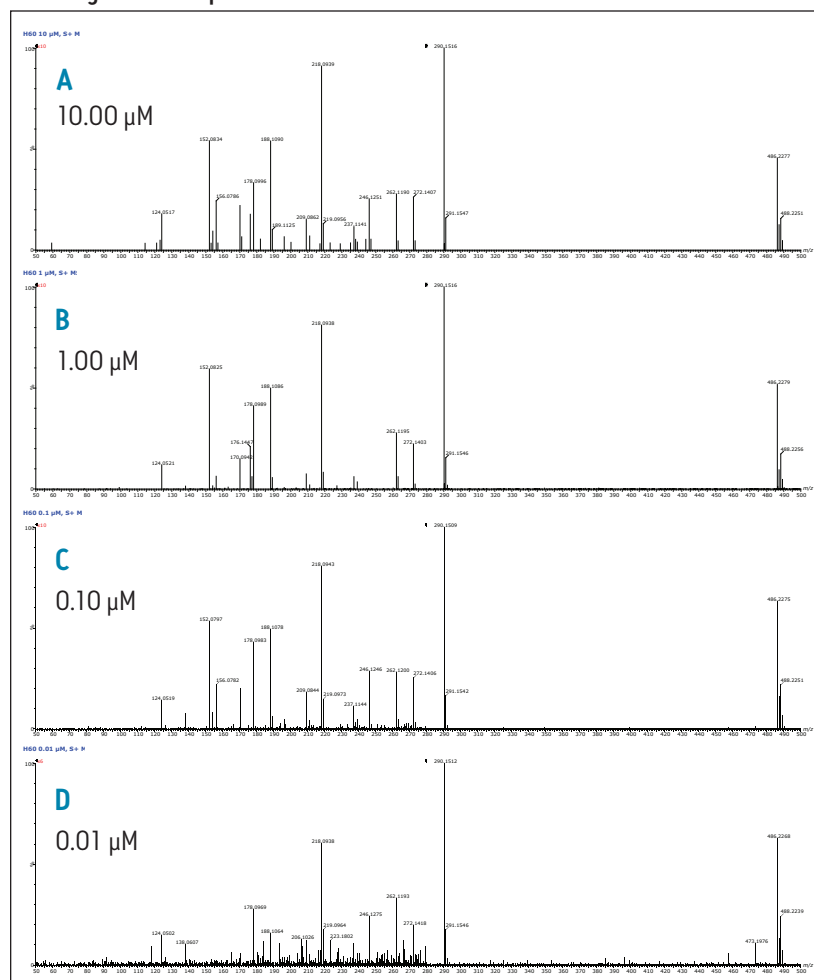


Figure 2. MS<sup>E</sup> high energy spectra of  $m/z$  486.227 at 1.46 min, a) 10.00, b) 1.00, c) 0.10, and d) 0.01  $\mu\text{M}$ .

Significant sensitivity gains for metabolite identification studies are achievable, as metabolites can be routinely detected and characterized at nM levels – providing more information from drug metabolism studies and giving insight into metabolism at physiologically-relevant concentrations.

The benefits of UPLC/SYNAPT G2-S and MetaboLynx XS v.2.0 described in this technology brief are now available as part of Waters industry-proven, class-leading workflow for metabolite identification. This workflow provides data independent analysis and chemically intelligent informatics that allow scientists to produce answers that can drive key decisions quicker than ever before.

# Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



Waters, UPLC, ACQUITY UPLC, and SYNAPT are registered trademarks of Waters Corporation. StepWave, MetaboLynx, and The Science of What's Possible are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2011 Waters Corporation. Produced in the U.S.A.  
May 2011 720003984EN LB-PDF

**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)