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アプリケーションノート

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

Waters Corporation

本書はアプリケーションブリーフであり、詳細な実験方法のセクションは含まれていません。

## Abstract

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

## Introduction

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 System coupled with a SYNAPT G2-S to enable confident metabolite identification analysis at

extremely low concentrations levels.

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

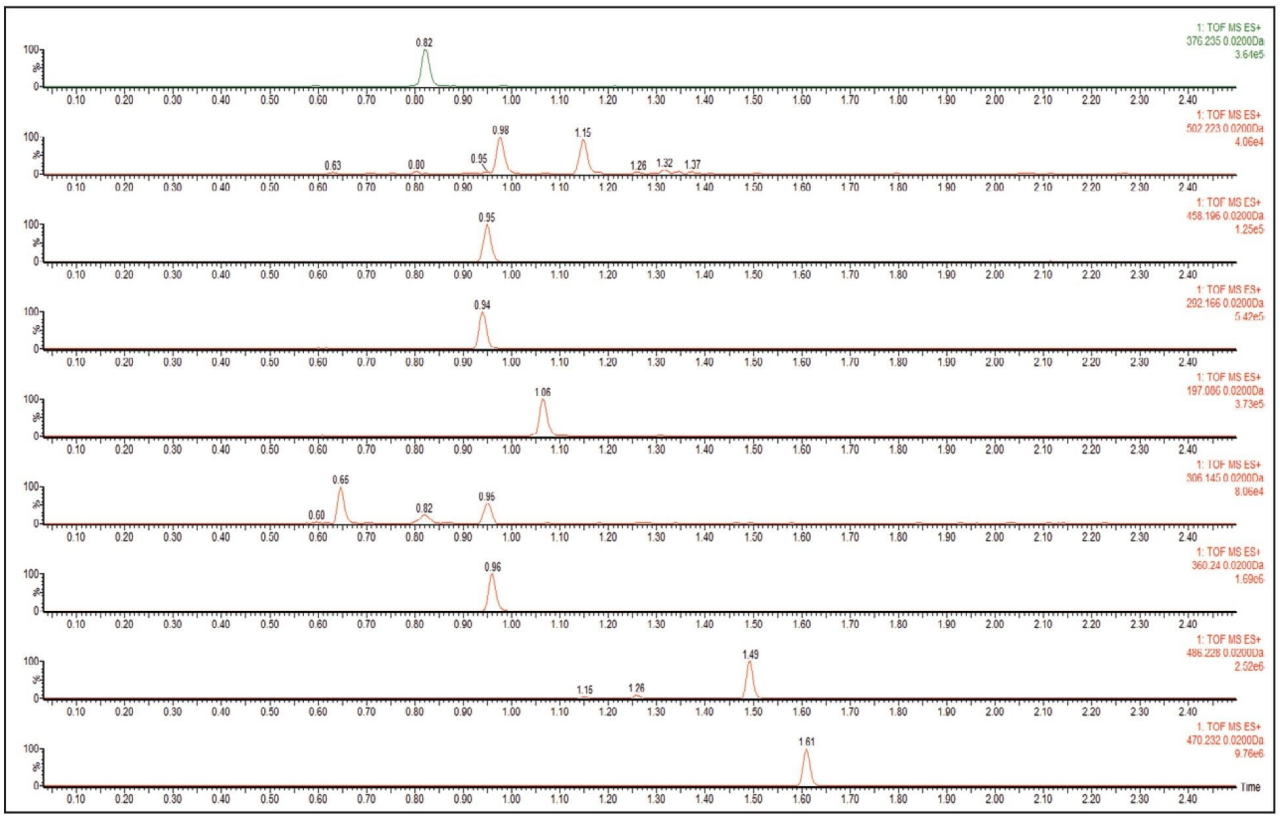


*Figure 1. Extracted ion chromatograms of metabolites at 0.01 µM.*

| | | | | | | Detection level (Incubation dilution, µM) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Metabolite name | % Area* | RT | *m/z** | theor | mDa* | 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 | |
| Parent-C15H19N3O2 | 2.7 | 1.06 | 197.0857 | 197.0845 | 1.2 | x | x | x | x | |
| Hydroxylation-C6H3Cl | 2.6 | 0.82 | 376.2352 | 376.2348 | 0.4 | x | x | x | x | |
| Hydroxylation | 1.3 | 1.26 | 486.2277 | 486.2272 | 0.5 | x | x | x | x | |
| Hydroxylation | 1.0 | 1.15 | 486.2281 | 486.2272 | 0.9 | x | x | x | x | |
| Hydroxylation + Deethylation | 0.8 | 0.95 | 458.1959 | 458.1959 | 0.0 | x | x | x | x | |
| 2xHydroxylation+Desaturation-C10H11N2Cl | 0.5 | 0.65 | 306.1462 | 306.1454 | 0.8 | x | x | x | x | |
| 2xHydroxylation | 0.3 | 0.98 | 502.2238 | 502.2221 | 1.7 | x | x | x | x | |
| 2xHydroxylation | 0.3 | 1.15 | 502.2227 | 502.2221 | 0.6 | x | x | x | | |

*Table 1. Area and mass accuracy values shown from 0.1 µM dilution series.*

## Results and Discussion

Human liver microsomes spiked with 10 µM 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 µL of sample were injected onto an ACQUITY UPLC BEH, 1.7 µm, 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 µM Nefazodone incubation and verified to be present (or absent) in four successive dilutions down to 0.001 µM (1 nM). These top 10 metabolites were detected down to 0.01 µM (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 µM dilution sample. Figure 2 shows the quality of $MS^E$ fragmentation data obtained for the hydroxy metabolite

(1.46 min) at successive dilutions down to the 0.01 µM dilution. Fragmentation data is clear and comprehensive down to 0.01 µM incubation levels, and structural assignments can be confidently made even at the lowest level shown.

$MS^E$ Fragment Ion Spectrum

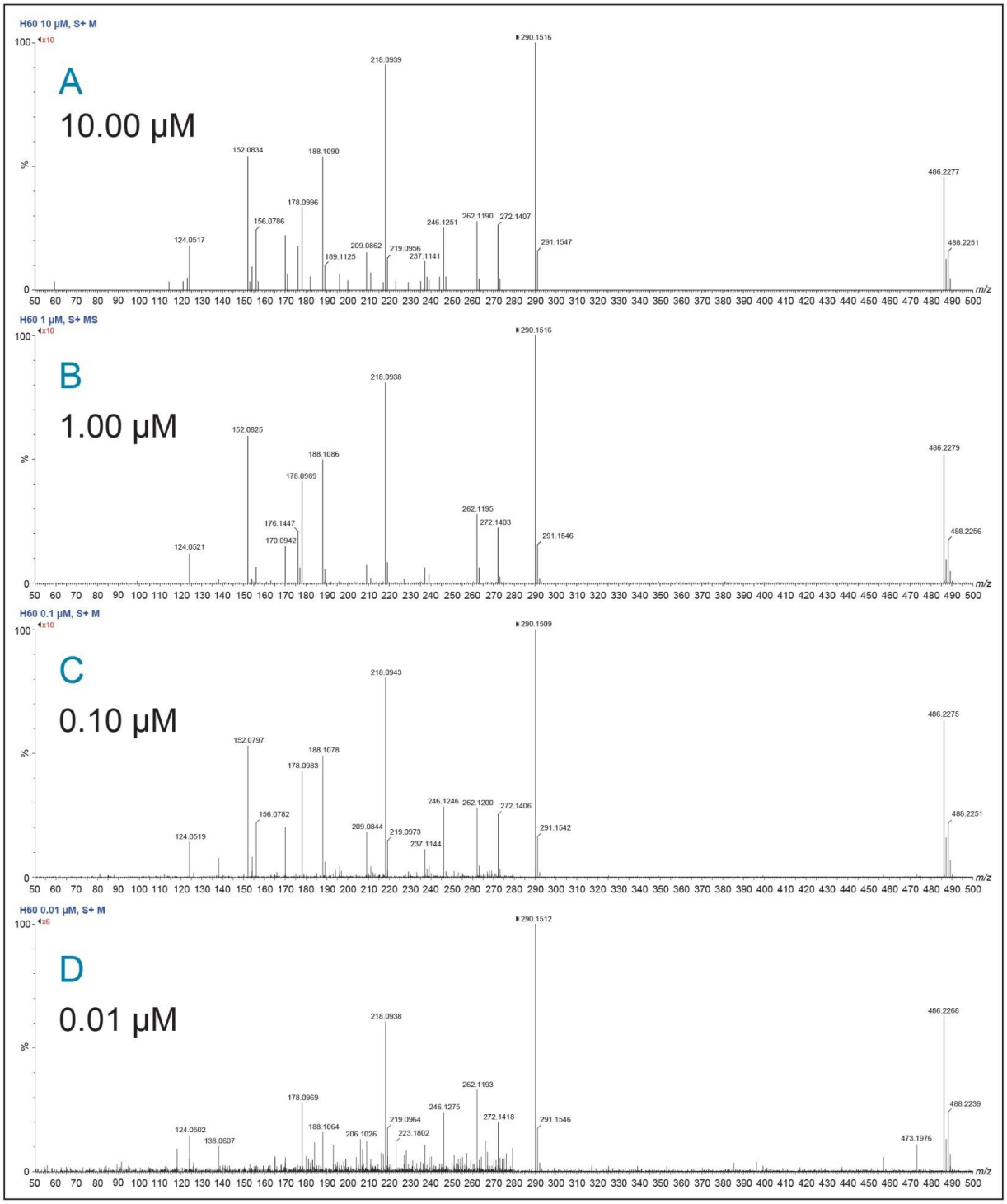


*Figure 2. MS$^E$ high energy spectra of m/z 486.227 at 1.46 min, a) 10.00, b) 1.00, c) 0.10, and d) 0.01 µM.*

## Conclusion

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

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MassLynx High-Resolution Applications <https://www.waters.com/nextgen/global/products/informatics-and-software/mass-spectrometry-software/masslynx-mass-spectrometry-software/masslynx-high-resolution-applications.html>

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