

## Complete Metabolite Identification of Nefazodone with SYNAPT G2-S and MetaboLynx XS v.2.0

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Waters Corporation

Dies ist ein Applikationsbericht, der keinen detaillierten Abschnitt zu Versuchen enthält.

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### Abstract

Detect and identify metabolites at much lower levels than any existing exact mass technique with SYNAPT G2-S and MetaboLynx XS v2.0.

### Benefits

- Detect and identify metabolites at much lower levels than any existing exact mass technique with SYNAPT G2-S and MetaboLynx XS v2.0

### Introduction

When performing metabolite identification, major metabolites are often easily detected using UPLC®/MS<sup>E</sup> analysis techniques. Workflows have been developed that accelerate the identification of these metabolites and have shown that throughput may be increased for metabolite identification by an order of magnitude or more. In early discovery, this type of throughput is paramount. However, speed is of less importance in biotransformation

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and development group processes, where the focus often shifts to thorough characterization of the sample – in these cases, the consequence of not detecting a minor metabolite may have severe repercussions. In light of increasing regulatory guideline pressures, such as Metabolites in Safety Testing (MIST) and targeting more complex therapeutic mechanisms, it has become exceedingly important to identify and characterize metabolite pathways fully in preclinical and clinical studies. In animal studies, metabolites that may only represent a small fraction of the total metabolic pathway may play a large role in human metabolism and toxicological relevance.

Their detection early in the preclinical process may provide huge advantages downstream in development timelines. In this technology brief we will describe a UPLC/SYNAPT G2-S experiment that redefines the limits of detection for these studies giving a more complete picture of metabolism than ever before.

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## Results and Discussion

Human liver microsomes spiked with 10  $\mu$ 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 Waters® SYNAPT G2-S Mass Spectrometer coupled with an ACQUITY UPLC® System. Data acquisition was performed with MS<sup>E</sup> in positive ion, sensitivity mode. 5  $\mu$ L of sample were injected onto an ACQUITY UPLC BEH, 1.7  $\mu$ m, 2.1 x 50 mm Column and run with a 10 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. A control comparison, chemically intelligent mass defect filters and MSE product ion analysis were used in order to provide evidence for each metabolite. Tabulated results for human microsomal met ID are shown in Table 1. The complete metabolic identification was reported for 10  $\mu$ M Nefazodone incubation. Figure 1 shows the three most intense and commonly identified +O metabolites, six additional +O metabolites can be identified from this sample. In Figure 2, all identified metabolites are shown. The insert shows the dynamic range necessary in order to identify the most to least abundant metabolites, with metabolite 502 at 3.75 e4 counts and 486 at 5.34 e7 counts; a difference of over three orders of magnitude in a single injection.

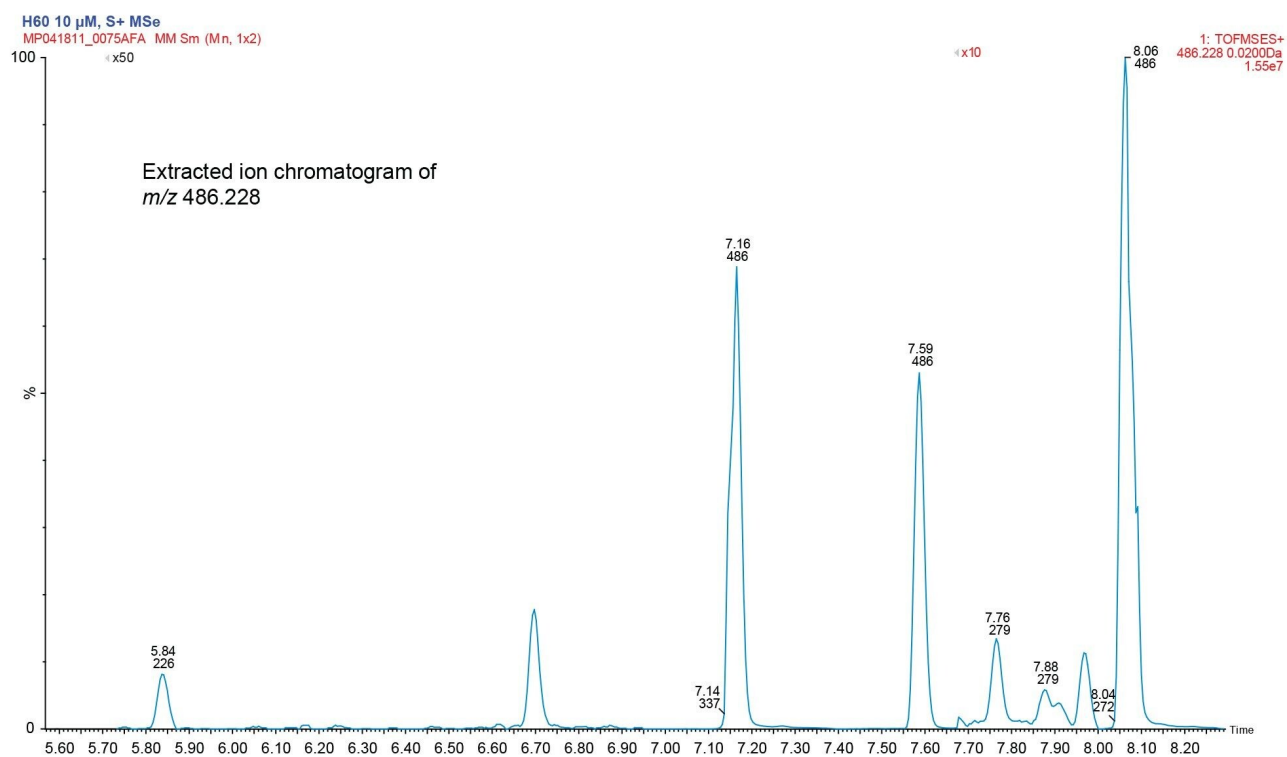


Figure 1. Extraction of +16 metabolites showing three expected hydroxylations and an additional six identified metabolites.

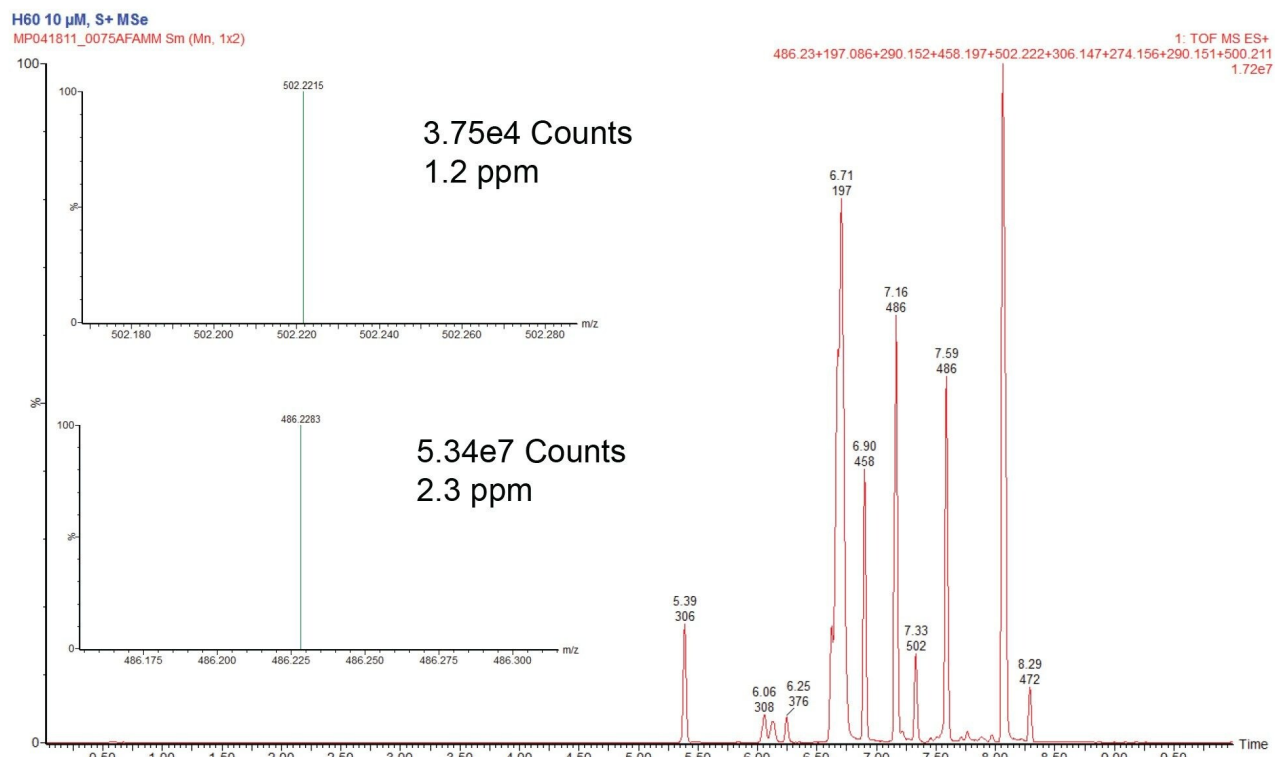


Figure 2. All identified metabolites.

| Proposed formula<br>change | Metabolite<br>formula | <i>m/z</i> | Mass error<br>(mDa) | Retention<br>time                                   |
|----------------------------|-----------------------|------------|---------------------|-----------------------------------------------------|
| -C15H19N3O2 (R_13)         | C10H13ClN2            | 197.0858   | 1.3                 | 6.71                                                |
| -C6H6 -C10H11N2Cl (R_10)   | C9H15N3O2             | 198.1253   | 1.1                 | 6.62                                                |
| +O -C15H19N3O2 (R_13)      | C10H13ClN2O           | 213.0798   | 0.3                 | 6.66                                                |
| -C13H17N2Cl (R_12)         | C12H15N3O2            | 234.1258   | 1.6                 | 6.29                                                |
| +O -H2 -C13H17N2Cl (R_12)  | C12H13N3O3            | 248.1049   | 1.4                 | 4.84                                                |
| +O -C13H17N2Cl (R_12)      | C12H15N3O3            | 250.1197   | 0.6                 | 6.12                                                |
| +O -C12H13N3O2 (R_11)      | C13H19ClN2O           | 255.1260   | -0.4                | 7.16                                                |
| +2O -H2 -C12H13N3O2 (R_11) | C13H17ClN2O2          | 269.1064   | 0.7                 | 5.50                                                |
| -H2 -C10H11N2Cl (R_10)     | C15H19N3O2            | 274.1561   | 0.6                 | 6.74                                                |
| +O -H2 -C10H11N2Cl (R_10)  | C15H19N3O3            | 290.1500   | 0.9                 | 6.06, 6.25,<br>6.68, 8.07                           |
| +O -C10H11N2Cl (R_10)      | C15H21N3O3            | 292.1669   | 0.8                 | 6.61                                                |
| +2O -H2 -C10H11N2Cl (R_10) | C15H19N3O4            | 306.1469   | 1.0                 | 5.39, 6.13, 6.62                                    |
| +3O -H2 -C10H11N2Cl (R_10) | C15H19N3O5            | 322.1405   | 0.2                 | 4.81                                                |
| -H2O -C6H3Cl (R_5)         | C19H27N5O             | 342.2280   | -1.4                | 6.56                                                |
| -CH2 -C6H3Cl (R_5)         | C18H27N5O2            | 346.2245   | 0.2                 | 6.31                                                |
| -C6H3Cl (R_5)              | C19H29N5O2            | 360.2387   | -1.2                | 6.88                                                |
| +O -CH2 -C6H3Cl (R_5)      | C18H27N5O3            | 362.2218   | 2.6                 | 6.21                                                |
| +H2 -C6H3Cl (R_5)          | C19H31N5O2            | 362.2537   | -1.9                | 5.75                                                |
| +O -C6H3Cl (R_5)           | C19H29N5O3            | 376.2338   | -1.0                | 6.03                                                |
| +2O -C6H6 -Cl+H (R_1)      | C19H27N5O4            | 390.2156   | 1.2                 | 5.73, 5.94                                          |
| -C6H6                      | C19H26ClN5O2          | 392.1871   | 1.8                 | 7.60                                                |
| +2O -C6H3Cl (R_5)          | C19H29N5O4            | 392.2299   | 0.1                 | 5.47                                                |
| -C6H4 (R_3)                | C19H28ClN5O2          | 394.2043   | 3.3                 | 7.43                                                |
| +O -C6H4 (R_3)             | C19H28ClN5O3          | 410.1979   | 2.0                 | 7.18                                                |
| -Cl+H (R_1)                | C25H33N5O2            | 436.2701   | -1.1                | 7.98                                                |
| +O -Cl+H (R_1)             | C25H33N5O3            | 452.2692   | 3.1                 | 7.24                                                |
| +O -H2 -C2H2 (R_0)         | C23H28ClN5O3          | 458.1974   | 1.5                 | 6.90                                                |
| +O -C2H2 (R_0)             | C23H30ClN5O3          | 460.2111   | -0.4                | 7.81                                                |
| -H2                        | C25H30ClN5O2          | 468.2193   | 2.7                 | 8.22                                                |
| +2O -Cl+H (R_1)            | C25H33N5O4            | 468.2626   | 1.5                 | 6.84                                                |
| Parent                     | C25H32ClN5O2          | 470.2336   | 1.3                 | 8.31                                                |
| +2O -H2 -C2H2 (R_0)        | C23H28ClN5O4          | 474.1908   | 0.3                 | 4.86, 6.27                                          |
| +O                         | C25H32ClN5O3          | 486.2289   | 1.1                 | 6.7, 7.16, 7.27,<br>7.31, 7.59, 7.76,<br>7.97, 8.09 |
| +2O -CH2                   | C24H30ClN5O4          | 488.2074   | 0.6                 | 6.22, 7.45                                          |
| +2O -H2                    | C25H30ClN5O4          | 500.2092   | 1.7                 | 7.22, 7.51, 7.89                                    |
| +2O                        | C25H32ClN5O4          | 502.2223   | 0.7                 | 6.71, 6.05, 7.33                                    |
| +3O -H2                    | C25H30ClN5O5          | 516.2009   | 0.8                 | 5.95, 6.14                                          |
| +3O                        | C25H32ClN5O5          | 518.2180   | 1.0                 | 5.50                                                |

Table 1. Identified metabolites of Nefazodone. Highlighted rows (shown in blue) are identified using mass error,

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*all other ID's are confirmed through isotopic pattern and fragment ion analysis.*

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## Conclusion

The advances presented in this technical brief allow the user to identify metabolites at much lower levels than any existing exact mass technique. Whether you're a CRO or a drug development lab Waters gives you the tools to perform complete qualitative and quantitative characterization of drug metabolism samples ensuring that the results generated can be presented with complete confidence. The benefits of UPLC/SYNAPT G2-S and MetaboLynx XS v.2.0 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 allows scientists to produce answers that can drive key decisions quicker than ever before.

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[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

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