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응용 자료

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

Waters Corporation

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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^E 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

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.

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 Waters® SYNAPT G2-S Mass Spectrometer coupled with an ACQUITY UPLC® System. Data acquisition was performed with MS^E 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 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 µM 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.

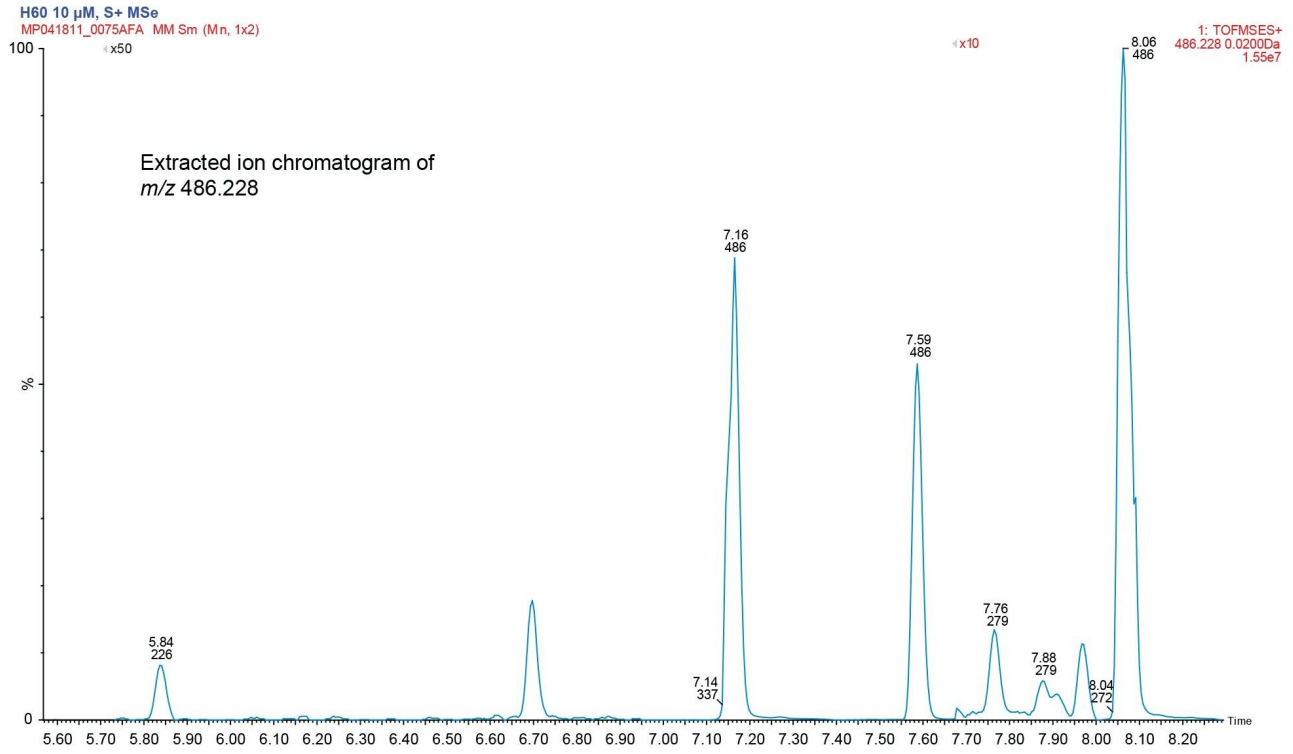

Figure 1. Extraction of +16 metabolites showing three expected hydroxylations and an additional six identified metabolites.

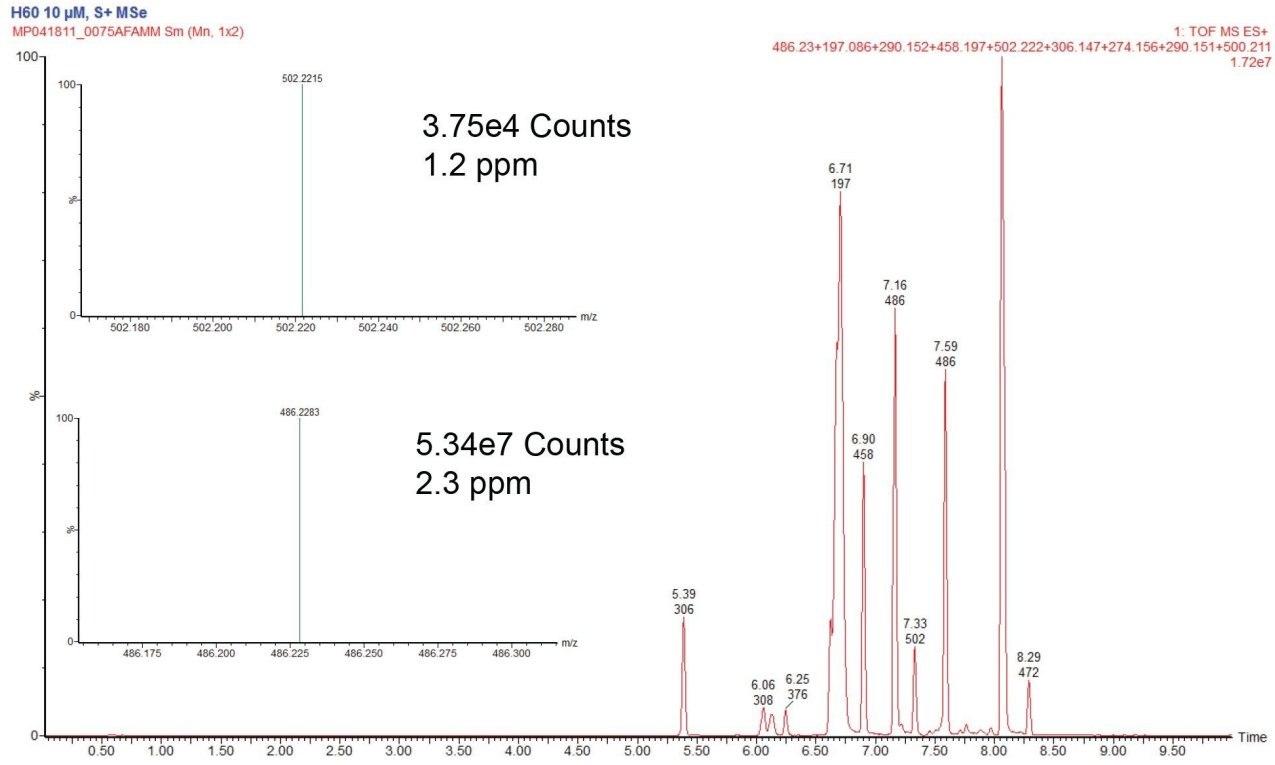

Figure 2. All identified metabolites.

Proposed formula change	Metabolite formula	*m/z*	Mass error (mDa)	Retention 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, 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, 7.31, 7.59, 7.76, 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,

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

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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