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Automated High Throughput UPLC-MS/MS Metabolite ID using MetaboLynx

Kate Yu, Jose M. Castro-Perez, Robert S. Plumb

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

In this application brief, we demonstrate the complete process of metabolite ID using MetaboLynx for an automated UPLC-MS/MS metabolite study.

Introduction

Metabolite identification is a critical and integral part of drug discovery and development process, and is particularly challenging due to its complexity and the level of expertise required. Liquid chromatography coupled with mass spectrometry (LC-MS) has become an important tool for metabolite ID. Tandem MS has been the major workhorse for metabolite ID for more than a decade, especially in drug discovery.

The study of metabolite ID using the tandem MS is a process that requires multiple analyte re-analysis.[1,2,3] A typical approach is to perform an initial LC-MS scan to identify any metabolites in the sample. Product ion scan is often employed to obtain the MS/MS information for each metabolite for structural elucidation. This is an effective approach, but it can be time-consuming because it is a multi-step process . It can also be demanding on the analyst's time.

The process of metabolite ID in modern drug discovery demands a rapid turn around time. Currently, the major bottle neck for metabolite ID studies is data processing and interpretation. In addition, for multistep analysis, the lack of automation can further increase the overall turnaround time; thus, there is a strong need to have a tool for rapid data processing, interpretation, and to automate the multi-step analysis.

The MetaboLynx Application Manager for MassLynx Software automates the process of peak detection, perform data interpretation, generate and execute method files for automated MS/MS verification of metabolite assignments, and presents an integrated final report for easy review.

In this application brief, we demonstrate the complete process of metabolite ID using MetaboLynx for an automated UPLC-MS/MS metabolite study. The example used here is an in vitro microsome incubation of Buspirone, a psychotropic drug with anxiolytic property. All sample analysis was performed using the Waters ACQUITY UPLC System coupled with the Quattro Premier XE Mass Spectrometer.

Waters ACQUITY UPLC System with the Waters Quattro Premier XE Mass Spectrometer.

Experimental

In-vitro Microsome Incubation

The parent drug buspirone was incubated separately with human and rat liver microsomes at 100 µM level. The incubation was at 37 °C, in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing the appropriate co-factors. The reaction was terminated after 90 minutes with 2 volumes of cold acetonitrile to 1 volume of sample. The samples were stored frozen at -20 °C and diluted 1:2 prior to UPLC-MS analysis. The control sample was prepared in the same way, except the cold acetonitrile was added before the enzymes and co-factors. As a result, the control sample contained the same matrix and components as in the incubated sample, but it did not have any metabolites formed.

LC Conditions

LC system:	ACQUITY UPLC System

Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm
Mobile phase:	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.1 % Formic acid

Gradient

Time (min)	Flow (mL/min)	%A	Curve
0.00	0.800	95.0	-
1.75	0.800	30.0	6
1.90	0.800	0.0	1
3.00	0.800	95.0	1

MS Conditions

MS system:	Quattro Premier XE Mass Spectrometer
Ionization mode:	Electrospray Positive
Capillary voltage:	3 kV
Cone voltage:	40 V
Source temp.:	130 °C
Desolvation temp.:	470 °C
Acquisition mode:	MS Full Scan Product Ion Scan

Results and Discussion

The incubated buspirone and control samples were analyzed by full scan UPLC-MS. Following data acquisition, MetaboLynx was used to process the data and results were visualized via the MetaboLynx browser, allowing results to be viewed and interpreted before selecting the metabolites of interest on which to perform MS/MS experiments.

The MS/MS experiment was set up automatically from the MetaboLynx browser and the MS/MS scan then performed using the same incubated sample. Once the analysis was completed, the results were incorporated into the previously created report to generate a single browser file. Then, structural elucidation was easily performed based upon the combined results.

Previous reports indicated that the major route of clearance for buspirone is CYP 3A4-mediated hydroxylation.[3,4]

The MS full scan result shown in Figure 1 shows six singly hydroxylated metabolites for buspirone at *m/z* 402, and several doubly hydroxylated metabolites at *m/z* 418. The "MSMS" column in the browser allows user to select the ions for further analysis by MS/MS scanning. The MSMS experiment setup wizard (Figure 2) allows the product ion scanning method and the sample list to be generated automatically. This process is performed only on ions whose intensities are above the user-defined threshold.

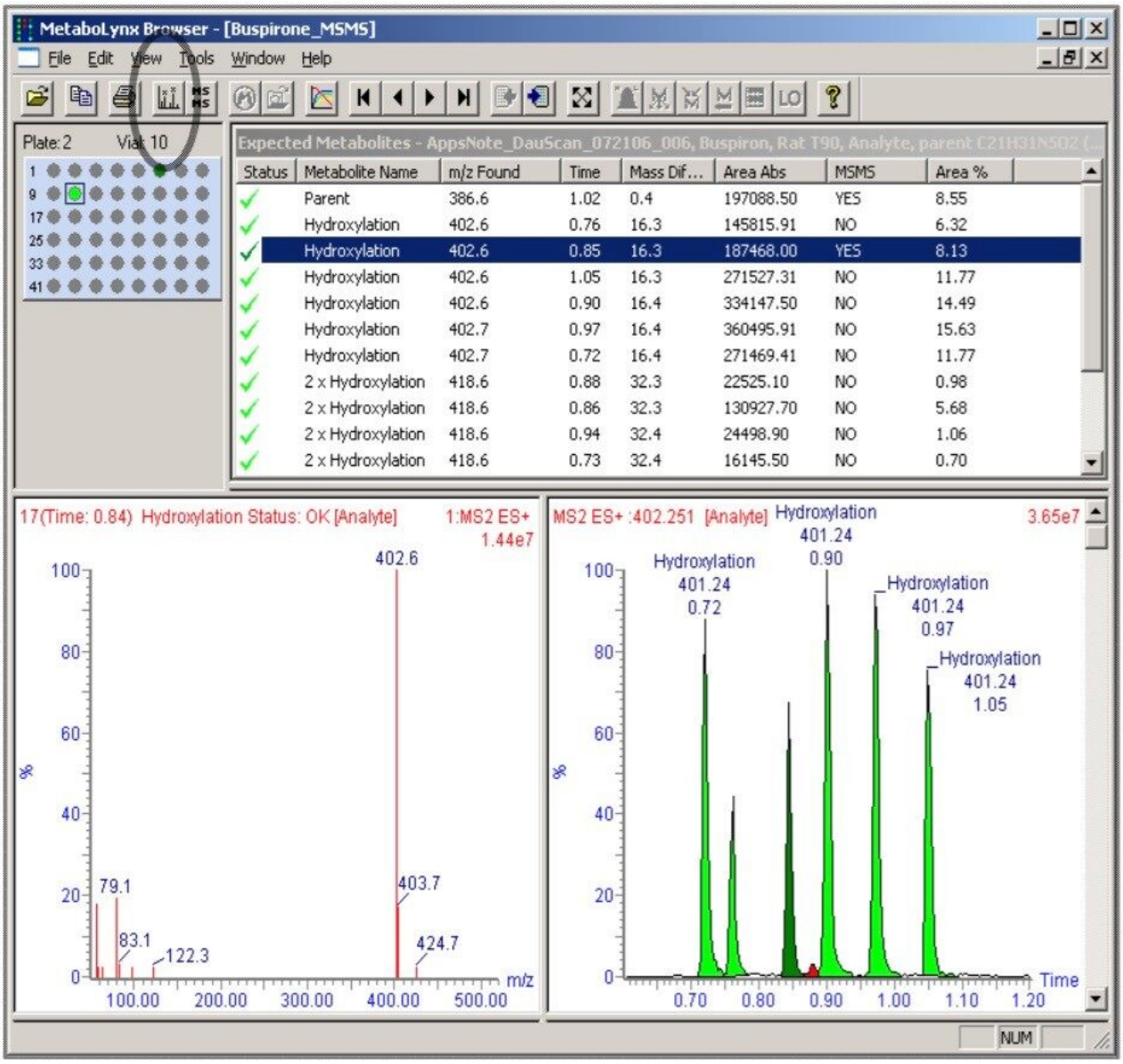

Figure 1. MetaboLynx full scan UPLC-MS report for the metabolites of buspirone.

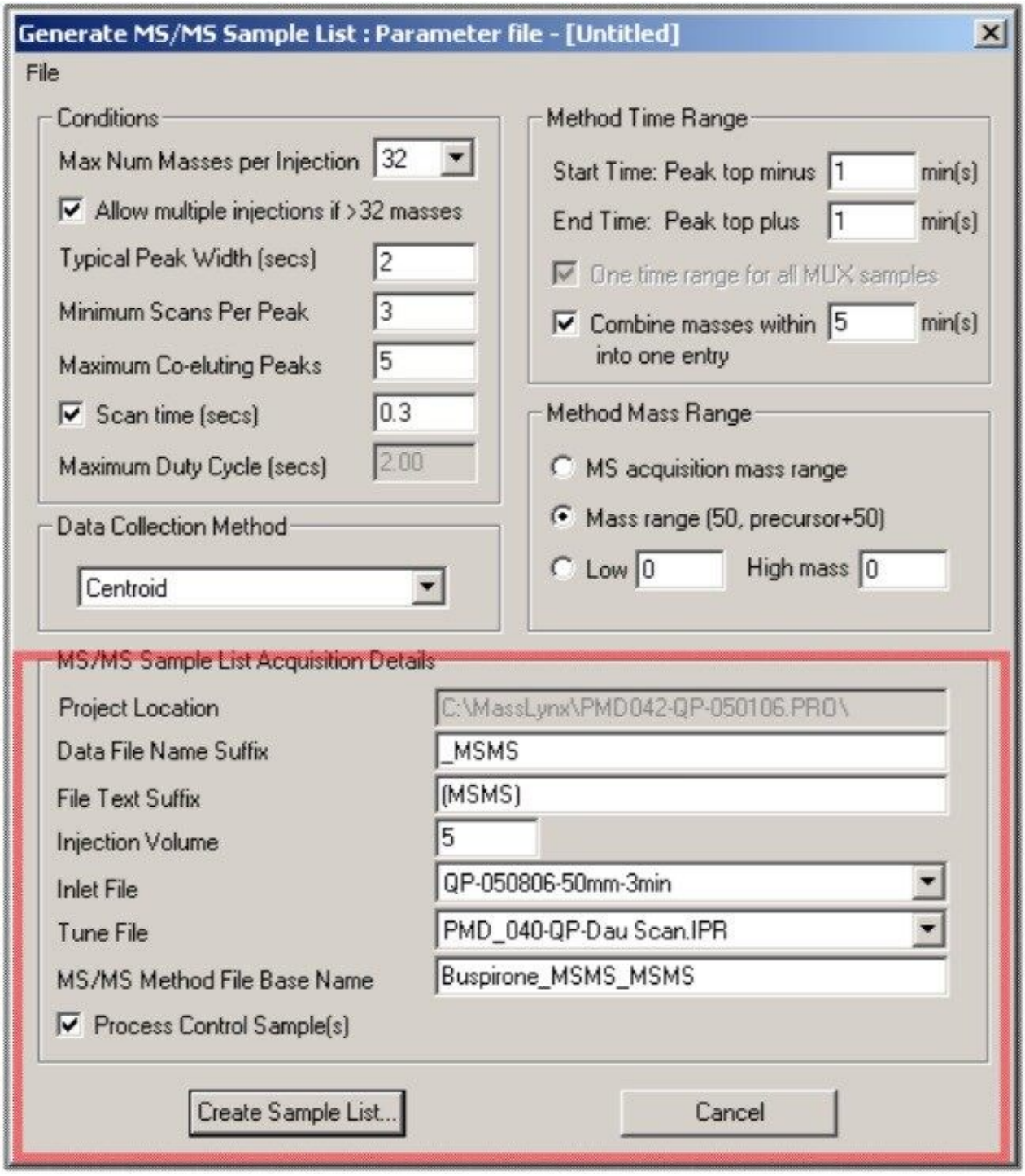

Figure 2. The MetaboLynx MSMS Experiment Setup Wizard.

Data obtained from the product ion scan were processed and combined with the original MetaboLynx report previously generated from the LC-MS full scan data. The MSMS results may be reviewed by clicking the MS/MS spectra button (circled in Figure 1) in the MetaboLynx browser.

The data was reviewed in the MSMS browser report (Figure 3), by clicking each metabolite that was identified on the list. The corresponding chromatographic peak in the TIC display window was highlighted in dark green (red arrow in Figure 3). The middle panel displayed the product ion spectrum of the parent drug and the corresponding metabolite.

In Figure 3, the metabolite with a retention time of 0.86 minutes was selected, and its corresponding MS/MS spectrum was displayed in the middle panel. When the hydroxylated metabolite MS/MS spectrum at retention time 0.86 minute was compared with the parent drug MS/MS spectrum, and the region of the molecule where the hydroxylation took place was easily discerned.

Figure 3. MetaboLynx MS/MS report for selected hydroxylated metabolites of Buspirone.

For the hydroxylated metabolite, the *m/z* 238 ion was 16 Da higher than the *m/z* 222 ion. Previous reports showed that *m/z* 222 is a major fragment of the parent drug following the loss of 1-pyrimidinylpiperazine (1–PP) moiety.[4] The appearance of the *m/z* 238 ion indicated that for this metabolite, the hydroxylation did not affect the 1–PP moiety.

To further compare the MS/MS spectra between the parent drug and the hydroxylated metabolite, a common fragment ion *m/z* 122 was observed (Figure 3). This supported the conclusion that the pyrimidine ring was unaltered for this specific metabolite (Figure 4)

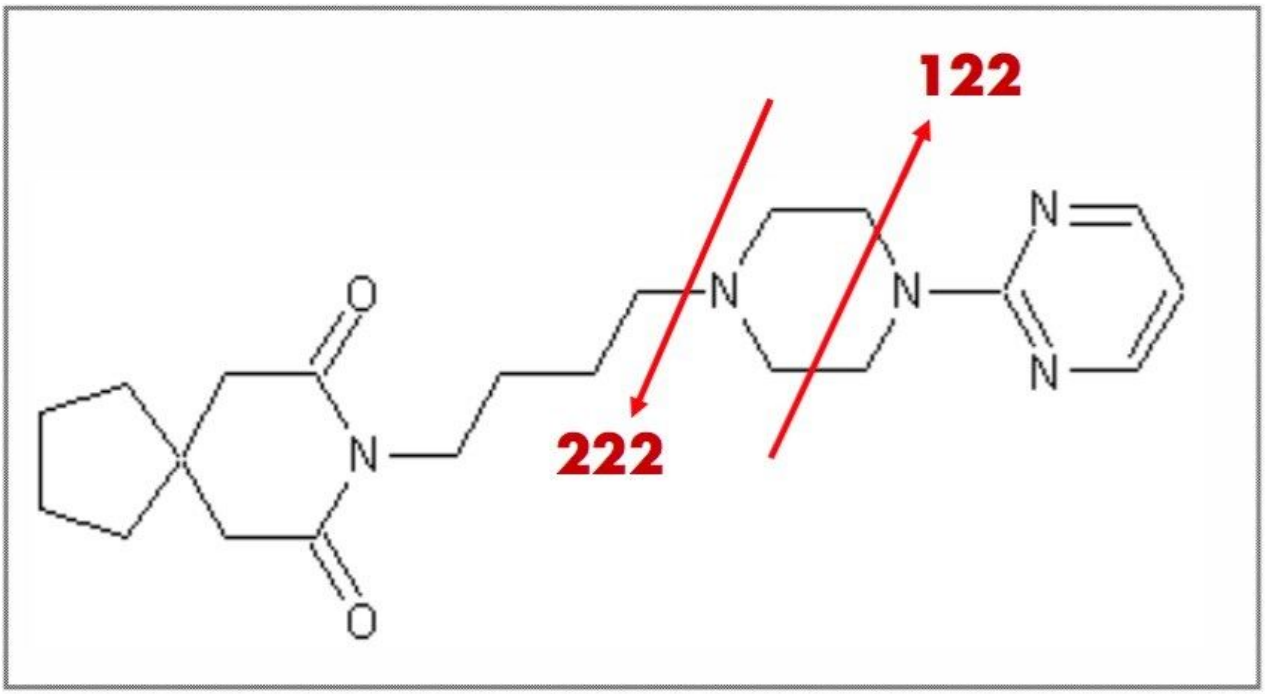

Figure 4. Proposed fragmentation pattern for buspirone.[4]

Conclusion

We have demonstrated the benefits of MetaboLynx for both UPLC-MS and UPLC-MS/MS metabolite detection and identification. In addition to providing fast data processing and result interpretation, MetaboLynx also automatically generates the MS/MS data acquisition list and incorporates the result into a single browser report. Combined with the superior results generated by the UPLC/Quattro Premier XE platform, MetaboLynx allows for easy data interpretation and fast structural elucidation with superior results.

References

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