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アプリケーションノート

High Throughput Metabolite Screening and Simultaneous Determination of Metabolic Stability using MetaboLynx

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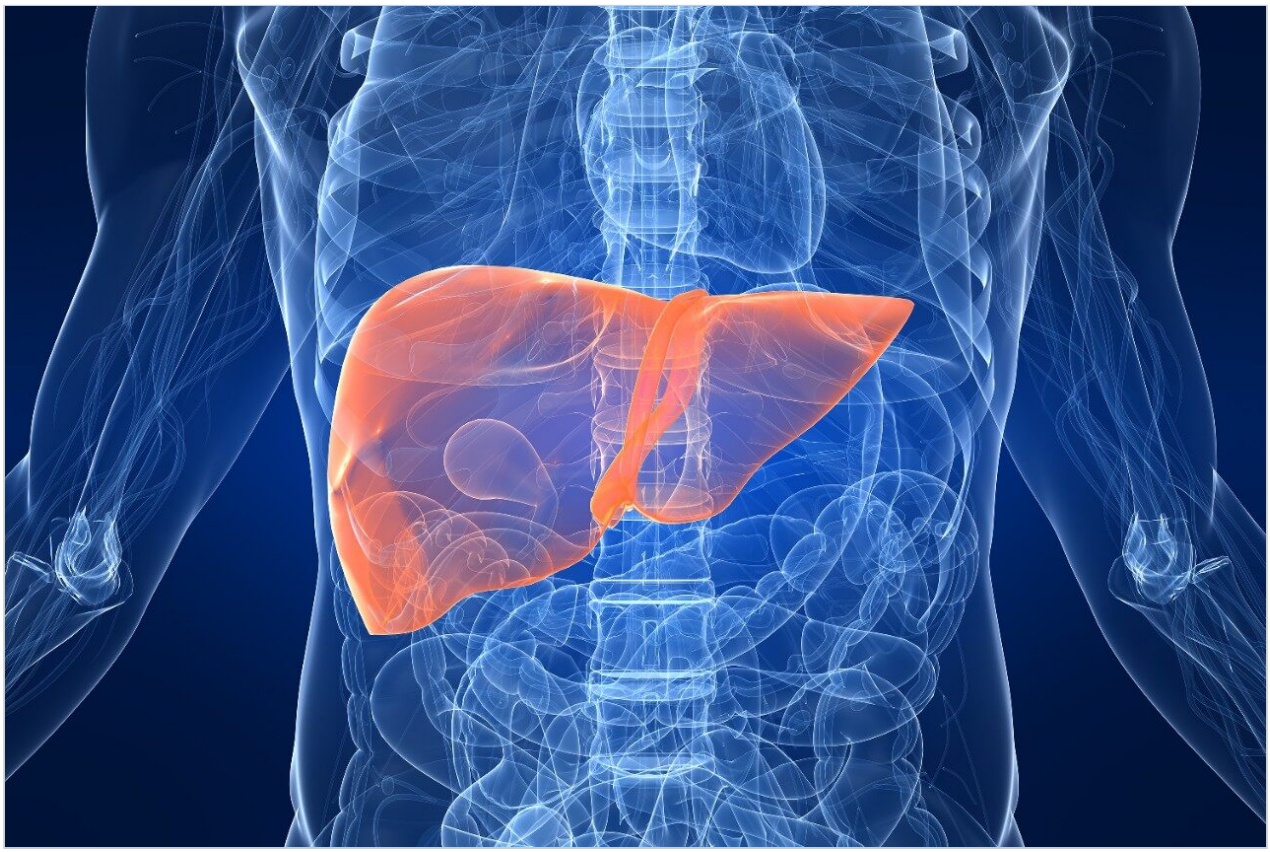

Abstract

In this application note, we demonstrate the process of metabolite identification with a simultaneous determination of metabolic stability using MetaboLynx.

Introduction

In modern drug discovery, data obtained from *in vitro* metabolism studies are used in a prospective manner to choose lead drug candidates. A promising drug candidate should possess commercially acceptable pharmacokinetic (PK) properties. Early metabolic information is useful to guide the decision-making process for structural modifications to achieve the desired PK properties. In addition, the ability to evaluate *in vitro* metabolic stability in human liver microsomes is useful for predicting the clearance and half-life in humans of new chemical entities.

For discovery metabolism studies, throughput remains one of the most critical factors to consider during method development. Metabolism data should be generated and interpreted rapidly; fast turnaround times allow chemists to incorporate the data into synthesis of the next compound series.

Sample throughput in drug metabolism can be increased in two stages:

- Reducing instrument analysis time with Waters UltraPerformance LC (UPLC) technology, leveraging columns packed with sub-2 µm hybrid materials to retain the practicality and principles of HPLC separations while increasing analytical speed, sensitivity, and resolution
- Reducing the data processing and interpretation time with the help of MassLynx Software’s MetaboLynx XS Application Manager

Data processing and interpretation has always been a major bottleneck for drug metabolism studies. MetaboLynx is a software tool that automates the process of peak detection and data interpretation. Results are presented in a browser with an easy-to-review format.

In addition, if the incubation was performed with multiple time points, MetaboLynx also automatically performs the metabolic stability calculation while performing the automated peak detection and data interpretation. This metabolic stability information is embedded within the MetaboLynx browser for easy review.

In this application note, we demonstrate the process of metabolite identification with a simultaneous determination of metabolic stability using MetaboLynx. The example used here is an *in vitro* microsome incubation of buspirone. All sample analyses were performed by using the ACQUITY UPLC System with the Quattro Premier XE Mass Spectrometer (Figure 1).

Figure 1. The ACQUITY UPLC System with the Quattro Premier XE Mass Spectrometer for UPLC-MS analysis.

Experimental

In vitro Microsome Incubation

The parent drug buspirone was incubated with human and rat liver microsomes at a 100 µM level. The incubation was carried out at 37 °C, in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing the appropriate cofactors. The reaction was terminated with two volumes of cold acetonitrile to one volume of sample. The samples were stored frozen at -20 °C and diluted 1:2 ratio prior to UPLC-MS analysis.

UPLC Conditions

LC System:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH C_{18} 1.7 µm, 2.1 x 150 mm
Column temp.:	90 °C
Mobile phase A:	Water + 0.1% Formic acid
Mobile phase B:	Acetonitrile + 0.1% Formic acid

Gradient

Time(min)	Flow(mL/min)	%A	Curve
0.00	0.800	95.0	
5.25	0.800	30.0	6
5.70	0.800	0.0	1
8.00	0.800	95.0	1

MS Conditions

Mass spectrometer:	Waters Quattro Premier XE
Ionization mode:	Electrospray positive
Capillary voltage:	3 kV
Cone voltage:	40V
Source temp.:	130 °C
Desolvation temp.:	470 °C
Acquisition mode:	MS full scan

Results and Discussion

A UPLC-MS full-scan experiment was performed for the incubated samples to obtain an initial screening of the metabolites. Figure 2 shows the extracted ion chromatograms of buspirone (*m/z* 386), hydroxybuspirone (*m/z* 402), and dihydroxybuspirone (*m/z* 418).

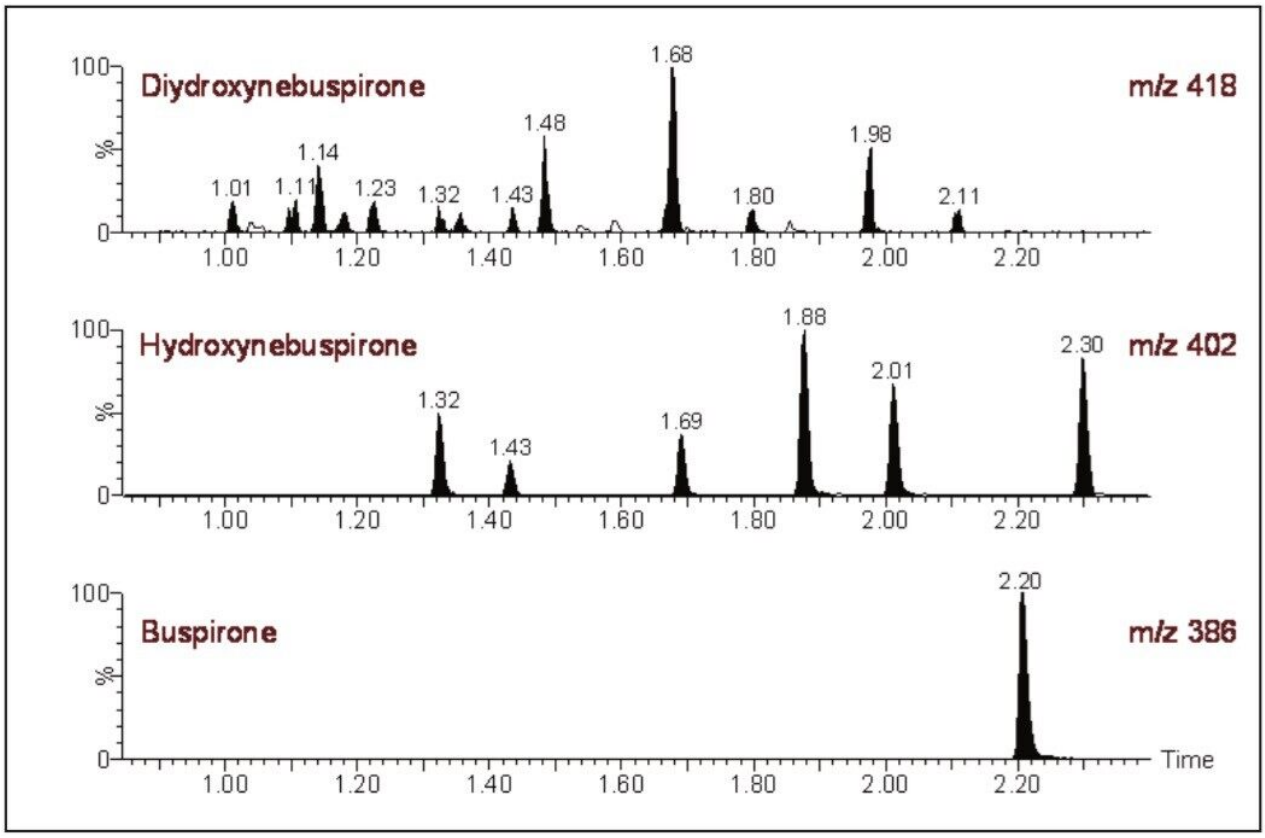

Figure 2. Selected ion chromatograms of buspirone and its metabolites obtained from the high temperature UPLC-MS full scan.

Previous reports[1, 2] have indicated that buspirone metabolites include six hydroxybuspirone and as many as eight dihydroxybuspirone. Results displayed in Figure 2 showed six hydroxybuspirone plus at least 13 dihydroxybuspirone. Further experiments are required to positively confirm the dihydroxybuspirones (beyond scope of this application note).

A complete report of the UPLC-MS full-scan experiments can be reviewed in the MetaboLynx browser, as shown in Figure 3.

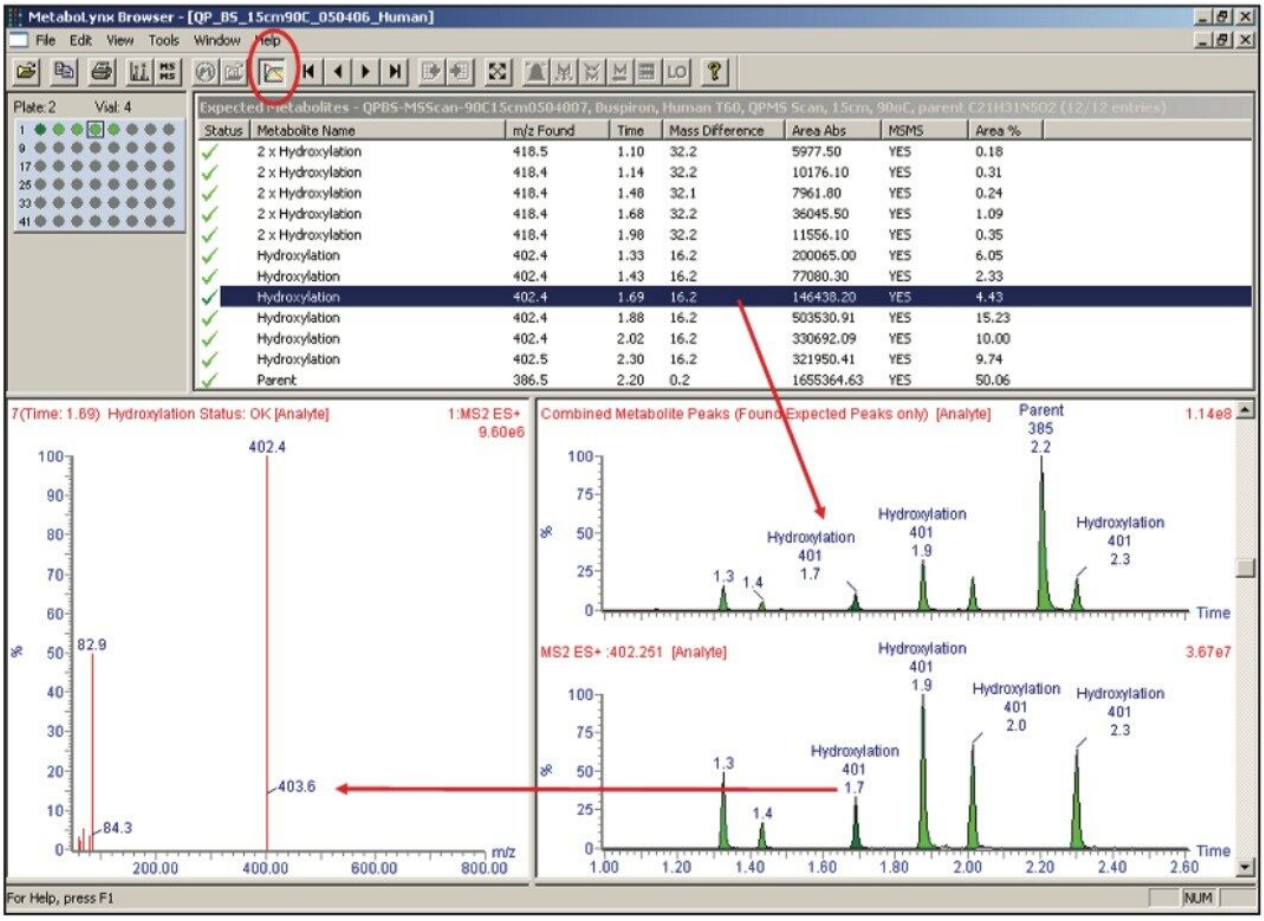

Figure 3. The MetaboLynx browser display of the LC-MS full-scan result for the buspirone incubated samples. The red circle highlights the metabolic stability button.

In Figure 3, the upper-left corner displays the plate map with the location of samples injected. In this example, vial 1 (dark green) was the control sample, and vials 2 to 5 contain the incubated samples at incubation time points of 15 min, 30 min, 60 min, and 90 min.

The top panel displays the list of identified metabolites. The bottom-right panel displays chromatograms. Of the two chromatograms shown here, the upper chromatogram is the combined trace of all identified metabolites plus the parent drug. Each peak is labeled with the metabolite’ s name, formula weight, and retention time. The lower chromatogram is the selected ion chromatogram of the hydroxybuspirone at *m/z* 402. The bottom-left panel shows the spectrum that corresponds to one of the hydroxylated metabolites of buspirone. This specific example shows the spectrum of the hydroxybuspirone with retention time of 1.7 min.

By clicking on the metabolic stability button (circled in red in Figure 3), a separate window is opened to display the metabolic stability results (Figure 4).

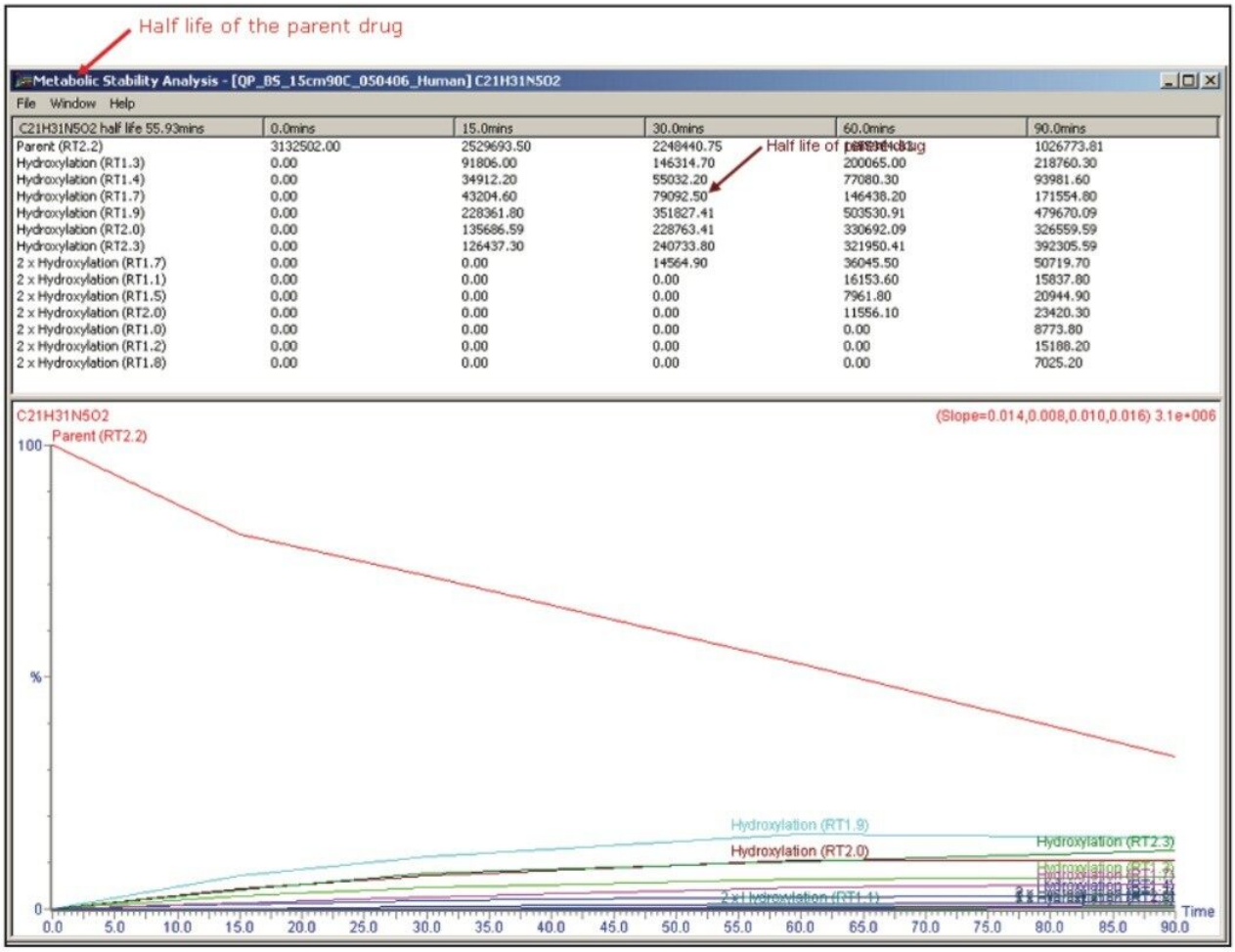

Figure 4. A screen capture of the rate of disappearance of buspirone and the rate of appearance of its metabolites.

The top panel in Figure 4 lists all of the identified metabolites, including the peak area of each metabolite at each time point. The half-life of the parent drug was calculated and shown on the top of the panel. In this example, the half-life for buspirone was 56 min. The bottom panel shows the plot of the disappearance for the parent drug as well as the appearance for metabolites.

Conclusion

We have demonstrated an entire information workflow that uses MetaboLynx to automatically perform data processing, result interpretation, as well as report generation. By performing a single UPLC-MS injection for each sample, we were able to obtain multiple levels of information. This includes the identification of potential metabolites, calculation of the metabolic stability such as half-life of the parent drug, as well as

the rate of appearance of the metabolites.

As a result, the significant time-savings reaped by both the fast and complete UPLC-MS analysis as well as the resulting streamlined data analysis enables scientists to be more adept as they make decisions about the metabolic stability of subsequent compound synthesis in these high-throughput screening drug discovery activities.

References

1. Kerns E, Rourick R, Volk K, Lee M. *J Chromatogr. B*. 1997; vol 698: 133.
2. Mingshe Zhu, Weiping Zhao, Humberto Jimenez, Donglu Zhang, Suresh Yeola, Renke Dai, Nimish Vachharajani, and James Mitroka. *DMD*. 2005; 33:500-7.

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