

HIGH THROUGHPUT METABOLITE SCREENING AND SIMULTANEOUS DETERMINATION OF METABOLIC STABILITY USING METABOLYNX

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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 μ M 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 μ m 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).

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).

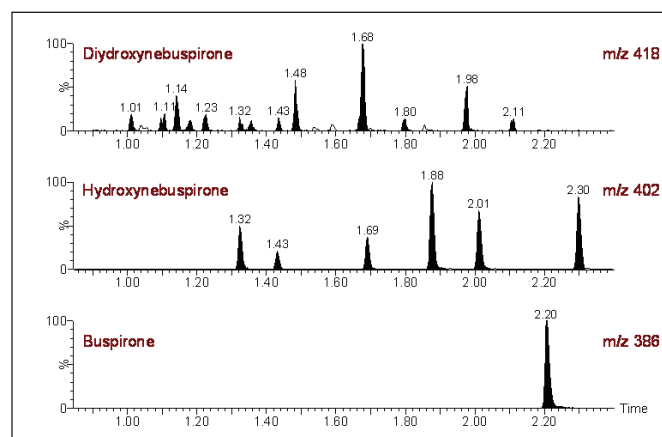


Figure 2. Selected ion chromatograms of buspirone and its metabolites obtained from the high temperature UPLC/MS full scan.

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

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

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

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