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High Throughput Metabolite ID Using Precursor Ion Scan and Neutral Loss Scan by UPLC-Tandem MS

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Abstract

This study is to demonstrates the utility of the ACQUITY UPLC System/Quattro Premier XE Mass Spectrometer platform in precursor ion scan and neutral-loss scan mode for high throughput *in vitro* metabolite identification studies, using buspirone as the test compound for this study.

Introduction

The application of metabolite identification studies in drug discovery is an active and growing field. Early feedback about metabolically labile sites or potentially toxic metabolites is crucial to direct compound design. *In vitro* metabolite studies are commonly used by many discovery programs to obtain an early picture of the metabolic fate of the drug. The major challenges for *in vitro* metabolite characterization in drug discovery are the demand of fast turnaround times and the lack of radio-labeled standards.[1]

Liquid chromatography coupled with mass spectrometry (LC-MS) has been the platform of choice for metabolite characterization. Amongst various mass spectrometers, Tandem MS has been the cornerstone of metabolite identification for more than a decade, due to of its relatively low cost, ease of use, and flexibility. These type of instruments are capable of performing a variety of data acquisitions including product ion, precursor ion, and neutral-loss scanning. The precursor ion scan and neutral-loss scan are commonly used in early drug discovery, because they provide a large amount of drug-related information.

In recent years, tremendous efforts have being made to reduce instrument analysis times in order to meet the demands of drug discovery and development. The strong demand for improving analytical throughput in drug discovery and development has resulted in technology advancements to reduce LC run time. One such advancement is UltraPerformance LC, which retains the practicality and principles of HPLC separations, while increasing the attributes of speed, sensitivity, and resolution. The ACQUITY UPLC System employs LC columns that incorporate sub-2 µm hybrid packing materials. As a result, a UPLC separation can be completed in less than a minute, while generating peaks of less than a second wide at half height. So, any paired tandem mass spectrometer must be able to scan at high speeds without loss of sensitivity or resolution to ensure quality analytical results.

The Quattro Premier XE Mass Spectrometer is equipped with T-Wave collision cell technology[2,3] which facilitates rapid data collection without loss in sensitivity. The Quattro Premier XE delivers enhanced sensitivity and resolution for class-specific monitoring using precursor ion or neutral loss analysis at very high scan speeds. It is capable of scanning at high speeds (up to 5000 amu/sec), making it ideal for UPLC.

The purpose of this study is to demonstrate the utility of the ACQUITY UPLC/Quattro Premier XE platform in

precursor ion scan and neutral-loss scan mode for high throughput *in vitro* metabolite identification studies, using Buspirone as the test compound for this study.[4,5]

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 levels. The incubation was at 37 °C, in a solution of 50 mM potassium phosphate adjusted to pH 7.4 containing NADPH (1.7 mg/mL in stock solution A) and UDPGA (1.9 mg/mL in stock solution A). To initiate the incubation, 250 µL of stock solution A added into the incubation tube containing 750 µL of the parent compound solution. The reaction was terminated after 90 minutes with 2 volumes of cold acetonitrile to 1 volume of sample. The samples were then stored frozen at –20 °C and diluted to 1:2 prior to UPLC-MS analysis.

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 range:	400–700 amu
Scan time:	0.3 sec

Results and Discussion

The initial MS full scan can was used to provide a rapid assessment of the metabolic fate of a compound. Following this, both precursor ion and neutral loss scans were employed for structure elucidation. Figure 1 shows the selected ion chromatogram obtained from the MS scan. Figure 1a was obtained from the protonated molecule of the parent drug (*m/z* 386), and 1b represents the single hydroxylated metabolite (MH^+ at *m/z* 402). Here, six peaks were observed, indicating that there are six possible single hydroxylated metabolites. Previous reports have shown that the buspirone parent molecule loses the 1- pyrimidinylpiperazine (1–PP) moiety, giving a fragment ion at *m/z* 222.[4,5] Therefore a precursor ion scan of the *m/z* 222 ion will help to identify the sites of hydroxylation.

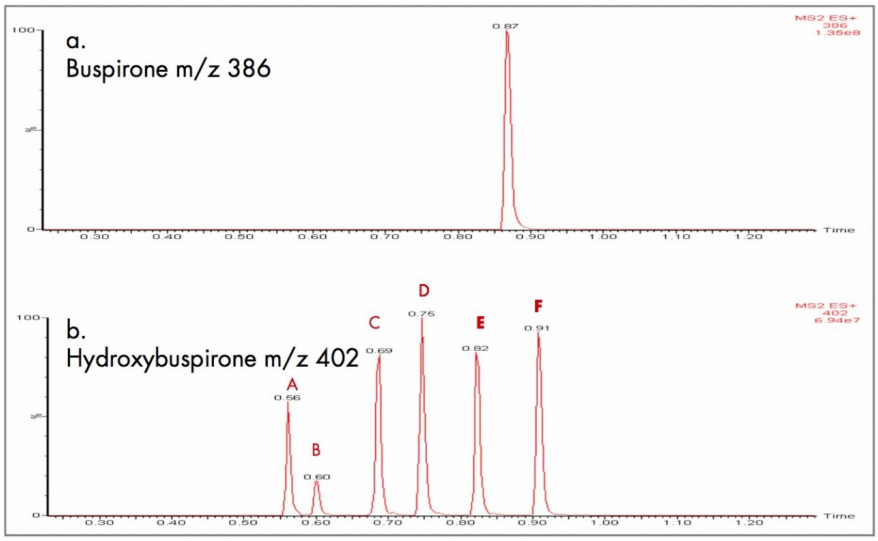

Figure 1. (a) Selected ion chromatogram of the parent drug buspirone at *m/z* 386. (b) Selected ion chromatogram of the single hydroxylated buspirone at *m/z* 402.

Precursor ion scanning is a powerful approach, since the only knowledge required is the fragmentation pattern of the precursor ion. Searching for common fragment ions can also provide vital information about the putative structure of a metabolite. By fixing the third quadrupole Q3 on selected ion, and scanning the first quadrupole Q1 over an appropriate range, the resulting spectra contain all of the precursor ions that produce the common fragment ion selected at Q3. In Figure 2, 2a shows the Total Ion Chromatogram (TIC) obtained from the precursor ion scan at *m/z* 222, and 2b and 2c show the corresponding spectra of the chromatographic peak 1 and peak 2, respectively. In 2b, there are three major peaks on the TIC with peak widths averaging at 1.8 second.

Peak 1 and 3 share similar spectra with the same base peak at *m/z* 402, indicating that they are the single hydroxylated metabolites. Peak 2 shows spectrum with the base peak at *m/z* 386 (Figure 2c), which is the parent drug. Peaks 1 and 3 also correlate to peaks E and F in Figure 1b. This suggests that for these two metabolites, the 1–PP region of the parent drug was hydroxylated.

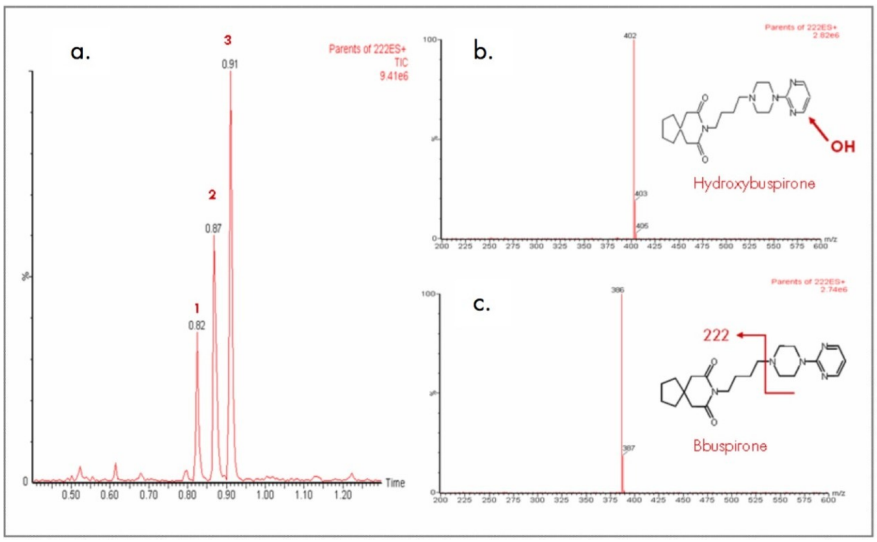

Figure 2. Result of the precursor ion scan of *m/z* 222: (a) shows the Total Ion Chromatogram (TIC) obtained from the scan, (b) shows the mass spectrum obtained from peak 3 (peak 1 shows very similar spectrum), and (c) shows the mass spectrum obtained from peak 2.

Constant neutral loss experiments require no prior knowledge of the parent compound. They can provide specific detection for the conjugated metabolites. For example, a neutral loss of 176 is the characteristic loss from the glucuronide metabolite.

Figure 3 shows the results obtained from the constant neutral loss scan (–176). The TIC (3a) has one major peak, indicating that there is one major glucuronide conjugated metabolite as a result of the incubation. Its corresponding spectrum is also displayed (3b).

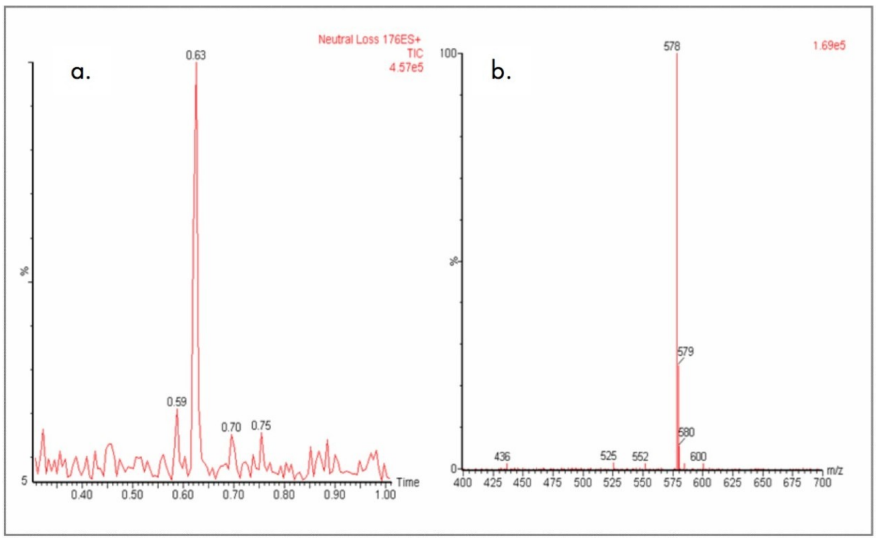

Figure 3. Results obtained from the neutral loss scan (176): (a) The total ion chromatogram and (b) the corresponding mass spectrum for the peak at 0.63 minutes, which correlates to the O-glucuronide metabolite of buspirone.

Conclusion

The use of the ACQUITY UPLC System combined with tandem mass spectrometry provides a very fast and effective approach to metabolite identification. The key advantage of UPLC is its ease of use and speed of analysis without the loss of chromatographic resolution. As demonstrated, coupling this breakthrough technology with a fast-scanning benchtop tandem quadrupole MS such as the Quattro Premier XE provides excellent levels of detection and speed of analysis.

References

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