Application Note: 348

Identification of Phase I Biotransformation Products of Buspirone using Parent Ion Scanning Triggered Data Dependent MS/MS

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Key Words

- Finnigan™ Surveyor™ HPLC
- Finnigan TSQ Quantum Ultra™
- Drug Metabolism
- *In vitro*

Introduction

The identification of biotransformation products of a pharmaceutical is important in understanding its pharmacological and its potentially toxicological effects. In order to improve the efficacy of a pharmaceutical and minimize any toxic side effects, drug candidates are being screened earlier in the discovery process. By identifying the metabolites of a drug candidate, the compound can be modified to block metabolic active sites increasing its duration of action, or the structure can be altered to prevent the formation of toxic by-products.

One of the common means of identifying drug metabolites by LC/MS/MS is employing the selective scan modes of a triple quadruple mass spectrometer, namely the parent ion scan and the constant neutral loss scan. These tandem mass spectrometry (MS/MS) scan functions allow a selective means of identifying multiple unknown compounds that are structurally related to a known pharmaceutical within the same LC run. The specificity of the parent ion scan or constant neutral loss scan effectively filters out endogenous interference ions, thereby reducing the possibility of identifying false positives.

This application report demonstrates the ability of the Finnigan TSQ Quantum Ultra (Thermo Electron, San Jose, CA, USA) to identify phase I metabolites of buspirone generated *in vitro* using parent ion scan mode. In addition to the Finnigan TSQ Quantum Ultra's high sensitivity and selectivity for identifying buspirone metabolites, the duty cycle of the Finnigan TSQ Quantum Ultra is capable of Data Dependent™ product ion acquisitions of potential metabolites that provide important structural information, all on the chromatographic time scale.

Experimental Conditions

Chemicals and Reagents: HPLC grade acetonitrile and water were purchased from Burdick and Jackson (Muskegon, MI, USA). Formic acid (95-97%) was obtained from Aldrich (Milwaukee, WI, USA). Buspirone, sodium bicarbonate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, β-nicotinamide adenine dinucleotide phosphate (NADP) and tris (hydroxymethyl)-aminomethane (TRIS) were acquired from Sigma (St. Louis, MO, USA). All chemicals were used as received.

***In Vitro* Incubation:** Sprague-Dawley rat liver microsomes (In Vitro Technologies, Baltimore, MD, USA) were prepared in a TRIS buffered solution (pH 7.4). After heating the liver microsome solution and the NADPH regenerating solution containing the enzymatic cofactors to 37 °C, buspirone was added to the system to initiate metabolism. The reaction was quenched at 60 minutes by the addition of an equal volume of acetonitrile to the microsomal solution. The sample was centrifuged at 10,000 rpm for 10 minutes and the resulting supernatant was stored at -80 °C until analyzed by LC/MS/MS.

Sample Analysis: The *in vitro* buspirone incubation samples were analyzed with the Finnigan Surveyor HPLC system (Thermo Electron, San Jose, CA, USA). Sample vials were maintained at 5 °C in the autosampler tray to inhibit metabolite degradation. A 10 µL injection volume was used for all sample analyses. Chromatographic separations were conducted with a 150 × 2.1 mm BetaBasic™ 18 column packed with 5 µm particles (Thermo Electron, Bellafonte, PA, USA). A binary gradient was employed for elution of the buspirone metabolites, where 5% ACN + 0.1% formic acid (A) and 95% ACN + 0.1% formic acid (B) served as the mobile phase. The elution program started at 5% B and holds for a period of 1.0 min, then ramps linearly to 55% B at 16.0 min. After holding at 55% B until 19.0 min, the gradient ramps to 95% B at 20.0 min and remains until t=23.0 min. The column is restored to 5% B at 23.1 min and it is equilibrated for a period of 11.0 min, equating to a total cycle time of 34.1 min. The flow rate during the gradient was 0.20 mL/min, which was directed to the Finnigan Ion Max™ source without splitting. For the equilibration period, the LC flow rate was increased to 0.30 mL/min.

Selective detection of the phase I metabolites of buspirone was accomplished using parent ion scanning mode on the Finnigan TSQ Quantum Ultra. To minimize sample consumption and maximize efficiency, two parent ion scan functions were acquired concurrently within the same LC run at a scan rate of 400 u/s. Data Dependent full-scan MS/MS product ion scans were acquired once an ion was detected above a signal threshold of 2.5E4 in parent ion mode. Product ion scans were acquired from *m/z* 30 to 5 *m/z* higher than the ion detected in parent ion mode in 0.5 seconds. The maximum cycle time for the four scan events (e.g., two parent ion scans and two Data Dependent product ion scans) was 3.0 seconds, allowing multiple parent and Data Dependent product ion scans to be obtained across a typical LC peak.

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Samples were introduced to the ESI probe on the Finnigan Ion Max source without splitting the effluent from the LC column. Ion formation and detection was carried out in the positive ion mode. Additional mass spectrometer parameters for the LC/MS/MS analysis of the buspirone samples were as follows:

ESI Spray Voltage: +3750 V
Sheath Gas Pressure: 45 arbitrary units
Auxillary Gas Pressure: 3 arbitrary units
Ion Sweep Gas Pressure: 5 arbitrary units
Ion Transfer Capillary Temperature: 350 °C
Source CID Offset: 5 V
Q2 Pressure: 0.7 mtorr argon

The four diagnostic buspirone ions monitored during a parent ion scan segment and the respective collision energies are: *m/z* 122 (34 eV); *m/z* 138 (34 eV); *m/z* 168 (34 eV) and *m/z* 265 (32 eV). The Data Dependent full-scan MS/MS spectra were collected at the same collision energies as the parent ion scan event on which they were triggered. All spectra were acquired under unit resolution conditions.

Results and Discussion

The triple quadrupole mass spectrometer is well suited to screen for the presence of metabolites via the use of constant neutral loss and parent ion scan modes. These highly specific scan functions permit the identification of analyte ions yielding either a specific neutral loss or a known fragment ion moiety that forms during collisionally-induced dissociation (CID) in the second quadrupole (Q2). For the parent ion scan, Q3 is fixed at a certain *m/z* that is consistent with a structurally related fragment ion of a known compound (e.g., a pharmaceutical) while Q1 is continuously scanned. Hence, any ion passing through Q1 that dissociates to yield the fragment ion to which Q3 is set will be detected. Such a parent ion being transmitted by Q1 is likely structurally related to the original compound investigated.

Even though a parent ion or constant neutral loss scan is specific for identifying potential biotransformation products, it is still necessary to determine the site of modification. By switching scan modes on the chromatographic time scale from parent ion to product ion mode, the structurally informative fragment ions can be measured. Such Data Dependent scan events are easily implemented on the TSQ Quantum platform. Additionally, the rapid scan rates for both parent and product ion scan modes permit multiple events to be acquired over the width of a typical LC peak, while still maintaining unit resolution.

The metabolic biotransformation products of buspirone have been well characterized.[1-4] Buspirone produces many mono- and dioxidative products during phase I metabolism. The primary sites for oxidation are the pyrimidine and piperazine rings on one side of the buspirone molecule and the bicyclic spiro ring system on the other. The product ion spectrum of the $[M+H]^+$ of buspirone in Figure 1 displays two key fragment ions at *m/z* 122 and *m/z* 265. These ions are attributed to structurally informative product ions on each side of the buspirone molecule as highlighted in fragmentation pathway of Figure 2.

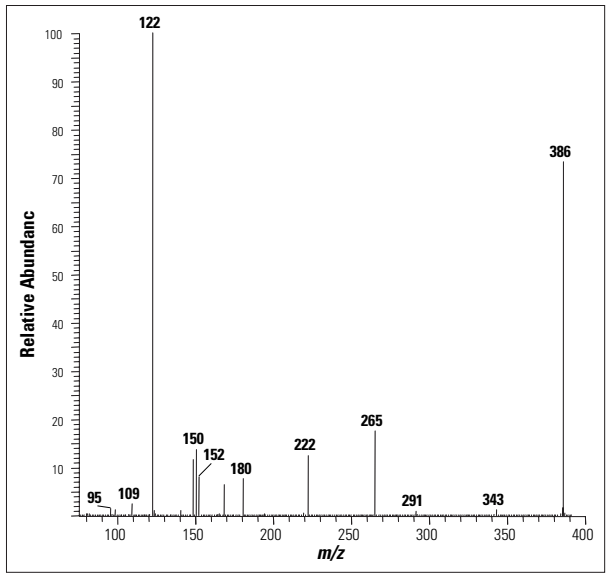

Figure 1: Full-scan MS/MS of $[M+H]^+$ of buspirone

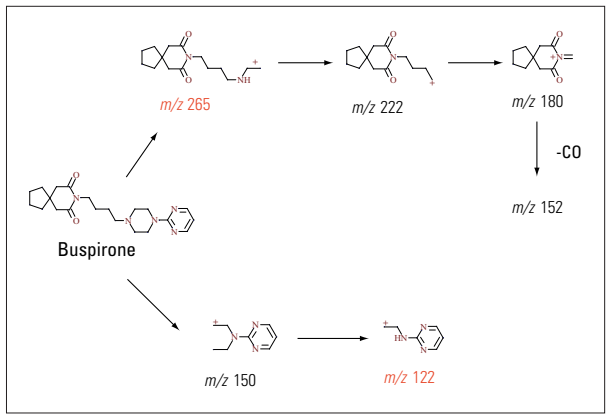

Figure 2: Fragmentation pathway for $[M+H]^+$ of buspirone

Implementing parent ion scans where Q3 is parked at the diagnostic ions of *m/z* 122 and 265 targets biotransformation products on each end of the molecule. Additional parent ion scans with Q3 set to *m/z* 138 and 168 will identify potential metabolites with at least one oxidation on each side of the buspirone molecule. Figure 3 displays the parent ion chromatograms for *m/z* 122, 265, 138 and 168 for buspirone incubated at a concentration of 10 µM for 60 minutes. The data in Figure 3 was acquired with only two LC runs, since two parent ion scan functions were collected within the same sample run. While the chromatogram monitoring the parents of *m/z* 122 is more information rich, multiple buspirone metabolites can be observed for all four parent ion scan segments.

Table 1 lists the proposed metabolites observed in the parent ion scan data from Figure 3. Some buspirone metabolites are not simple mono- or dioxidation products of buspirone (e.g., N,N-desethyl buspirone at t_R= 11.28 min.), making identification by using a more selective scan mode, such as multiple reaction monitoring (MRM), difficult or impossible. Yet, the Finnigan TSQ Quantum Ultra has a sufficiently high duty cycle and increased sensitivity using parent ion scan mode to identify nineteen metabolites and the original buspirone species at the 10 µM level, using only two sample injections without sample preconcentration or cleanup.

In addition to identifying the masses of potential buspirone biotransformation products, the Data Dependent product scans triggered from the parent ion survey scan helped in determining the site of modification. For example, six buspirone metabolites were observed with *m/z* 402 (see Table 1). Inspection of the product ion spectra for three of these putative monooxidation metabolites derived from the parent ion scans of *m/z* 122 indicates that these are three structurally different compounds (Figure 4). With the help of various literature references, comparison of these product ion spectra to that of buspirone (Figure 1) and accurate mass measurements of the product ions on the LTQ-FT (data not shown), these three components at retention times of 8.60, 10.58, and 12.93 minutes have been identified as oxa-buspirone, hydroxybuspirone and buspirone N-oxide, respectively. Their proposed structures and identifying fragment ions are shown in Figure 5.

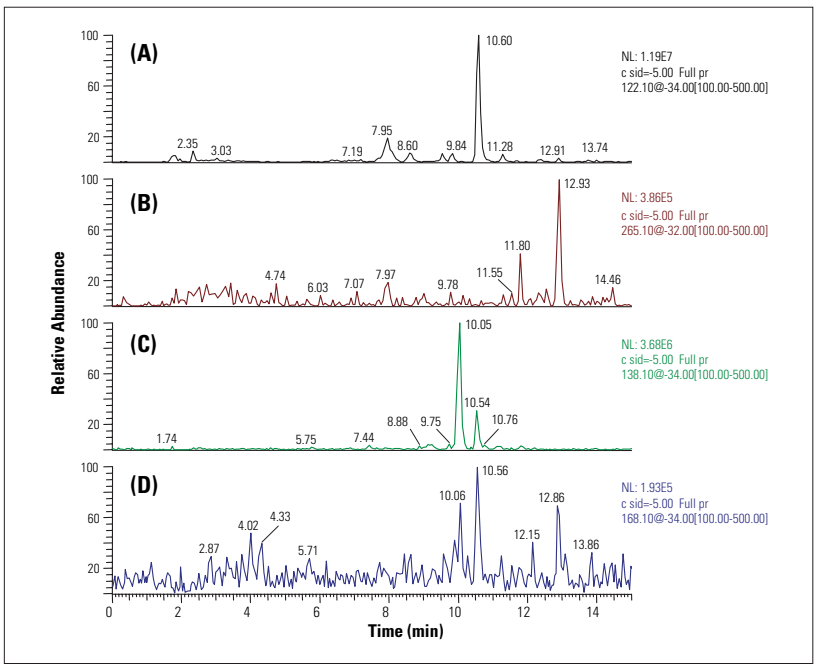

Figure 3: Parent ion chromatograms for (A) *m/z* 122, (B) *m/z* 265, (C) *m/z* 138 (D) *m/z* 168

			Parent Ion Survey Scan			
Retention Time (min)	**Metabolite [M+H]+**	**Proposed Structure**	**122**	**265**	**138**	**168**
2.35	165	1-pyrimidinylpiperazine	X			
6.37	418	Dihydroxybuspirone	X			
7.19	418	Dihydroxybuspirone	X		X	
7.44	418	Dihydroxybuspirone			X	
7.95	402	Hydroxybuspirone	X			
7.97	350	Guanidino Buspirone		X		X
8.07	418	Hydroxy Oxa-buspirone	X		X	
8.09	400	Oxo-buspirone	X			
8.60	402	Oxa-buspirone	X			
9.19	418	Dihydroxybuspirone			X	
9.53	376	N,N-desethyl Hydroxybuspirone	X			
9.84	402	Hydroxybuspirone	X			
10.05	418	Dihydroxybuspirone				
10.60	402	Hydroxybuspirone	X		X	X
10.76	376	N,N-desethyl Hydroxybuspirone		X		
11.18	418	Hydroxybuspirone N-oxide	X			
11.28	360	N,N-desethyl buspirone	X			
11.80	402	Hydroxybuspirone		X		
12.39	386	Buspirone	X	X		
12.91	402	Buspirone N-oxide	X	X		X

Table 1: Buspirone metabolites identified via parent ion scan mode on the TSQ Quantum Ultra

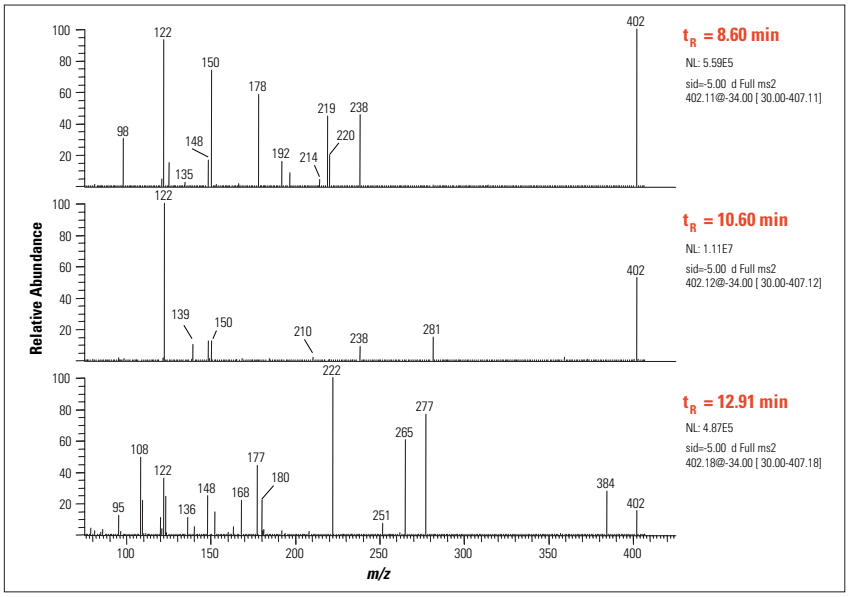

Figure 4: Data Dependent product ion spectra for three M+16 metabolites (*m/z* 402) of buspirone

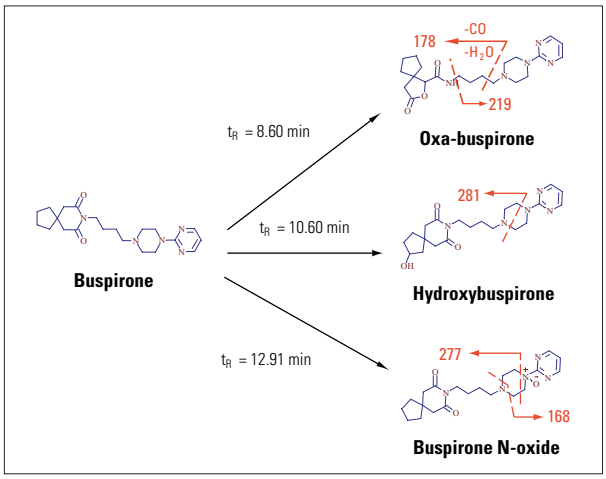

Figure 5: Proposed fragmentation pathways for three M+16 metabolites of buspirone

Conclusions

The Finnigan TSQ Quantum Ultra was able to identify nineteen phase I metabolites of buspirone generated *in vitro* using multiple parent ion scan functions. Data Dependent product ion spectra, triggered from the parent ion survey scans, were collected for the buspirone metabolite ions, thereby providing useful structural information on the LC time scale. In addition to the expected mono- and dioxidative phase I biotransformation products, dealkylation metabolites were observed, which would have been difficult to identify using a more selective scan technique (i.e., MRM).

References

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