Identification of Phase I and Phase II Metabolites of Buspirone on the Q TRAP™ LC/MS/MS System

Purpose
This application note describes the use of the new Q TRAP™ LC/MS/MS system with Metabolite ID software for Phase I and Phase II metabolite identification and discusses its unique capabilities that increase your productivity and confidence in your results.

Overview
Current trends in drug development are placing more emphasis on characterization of potential drug candidates earlier in the discovery process. The goal is to take more qualified drug candidates to pre-clinical testing and clinical trials. This shift in focus will enable drug researchers to save time and money. Nearly 60% of the costs to develop a new drug are incurred in the clinical trials phase, so there is much to be gained by selecting only the most promising drug candidates for testing at this level. Early discovery ADME (adsorption, distribution, metabolism, and excretion) studies are critical to predict a drug candidate’s success in further downstream testing.

Early assessment of drug metabolism of in vitro and in vivo samples provides initial information about a potential drug’s biotransformation and metabolite characterization. To obtain maximum characterization information, including chemical structure and quantity, LC/MS (liquid chromatography/mass spectrometry) is often employed because it has sufficient sensitivity and specificity to measure nanogram levels of metabolites in complex matrices. Ion trap mass spectrometers are widely used to identify metabolites, and triple quadrupole mass spectrometers are generally used to measure the amount, or concentration of metabolites detected.

Key Features of the Q TRAP System
- Exceptional sensitivity for metabolite identification and characterization
- Powerful advanced scan modes, including neutral loss and precursor ion scans, can be used in flexible combinations to achieve unprecedented selectivity
- Broad linear dynamic range provides true triple quadrupole quantitation performance and enhances identification of ions in complex matrices
- Information-rich MS/MS and MS³ spectra with important low mass structural ions provide confidence in results
- Metabolite ID application software makes it easy to go straight to the answers

Experimental Conditions
Rat liver microsomal incubate samples were injected on a Hypersil-Betasil C18 column (1 x 100 mm). Samples were eluted using Shimadzu 10 AD pumps with a gradient of water/methanol, from 10 to 90% methanol over 10 minutes at a flow rate of 70 µL/min. Direct injection (5 µL) on column was performed using a PE Series 200 autosampler. A TurboIonSpray® source was operated at 350° C on the Q TRAP system.
Results and Discussion
The new Q TRAP™ LC/MS/MS system combines superior ion trap capabilities with triple quadrupole performance to provide a new level of performance and versatility in LC/MS for metabolite identification. The patented hybrid linear design enhances sensitivity, resolution, and duty cycle for MS and MS/MS full scan modes, and combines these features with the linear dynamic range and scan functions of a triple quadrupole instrument.

Add Metabolite ID application software and get automated data acquisition and processing, with a range of options to ensure maximum productivity. Metabolite ID application software leverages the Q TRAP system technology and IDA to provide rapid, high-confidence results in the fewest number of experiments.

Metabolite ID:
- Identifies predefined and user-defined Phase I and Phase II transformations
- Detects unexpected metabolites by recognition of isotope ratio patterns
- Triggers automatic acquisition of MS/MS structural information
- Confirms metabolites by correlation comparison to parent drug
- Performs fully automated data acquisition and processing in batch mode
- Includes a powerful library search function
- Contains an easy-to-use browser for viewing results

In this study, the Q TRAP system was used to identify Phase I and Phase II metabolites of buspirone.

The Q TRAP system has the advantage of using highly selective scan functions such as neutral loss and precursor ion scans to facilitate detection of important biotransformation products from complex matrices. The structure and mass spectrum of buspirone (Figure 1) lead to the prediction that fragmentation that can occur on the A or B side of the structure can be identified using precursor ion scans at mass 122 and 168, respectively. Further information can be gained by using these two masses in combination with masses of expected transformations, i.e. precursor ion of 138 for hydroxylation occurring on the A side.

Figure 2 compares a normal full scan TIC trace from a liquid chromatography gradient run to a...
precursor ion scan of mass 122 and demonstrates the gain in selectivity. Up to two precursor ion scans can be combined in the Information Dependent Acquisition (IDA) survey step, minimizing the number of injections required to gain maximum information.

Figure 3 shows XIC traces of two metabolites that were identified with a precursor ion scan of mass 122 plus buspirone. An enhanced product ion (EPI) scan of a peak at 6.86 minutes from the XIC 402 trace confirms hydroxy-buspirone as a major metabolite and provides important information on the site of modification. The IDA method also contained an enhanced resolution scan that improves the monoisotopic mass assignment, as well as provides isotope ratio information for further confirmation (Figure 4).

The number of metabolites identified with the precursor ion scan method was compared to the number of metabolites identified with an enhanced MS method. Table 1 illustrates that the precursor ion scan method clearly identifies and confirms more metabolites in all cases.

For identification of Phase II metabolites, an IDA method with a neutral loss scan of 176 as the survey scan was used to selectively identify three glucuronide species of buspirone (Figure 5). Prior MS/MS experiments with buspirone glucuronides indicated the aglycon species as the major fragment ion, and subsequently, the IDA method was set to include an MS3 experiment following EPI, in order to gain more structural information. The MS3 experiment run on the aglycon metabolite (m/z 402) is much richer in structural information due to the triple quadrupole fragmentation pattern of the Q TRAP™ system. Figure 5 shows the resulting MS3 spectra of m/z 402 from the EPI scan, which, combined with supporting chemical structural information, clearly indicates the site of hydroxylation. This powerful automated approach to identifying and characterizing metabolites all in a single LC/MS IDA analysis can be used on expected and unexpected metabolites.
Conclusions

The Q TRAP™ LC/MS/MS system with IDA and Metabolite ID automates every aspect of metabolite identification and provides the highest quality results. Advanced, automated data processing and reporting puts high-confidence results at your fingertips in less time. The Q TRAP LC/MS/MS system, a hybrid linear ion trap, provides superior ion trap performance and high-performance triple quadrupole functionality. Scan functions unique to ion trap MS, such as precursor ion and neutral loss scans, simplify the task of finding metabolites in complex matrices, and increase the amount of information gained per experiment. Phase I and Phase II metabolites can be identified, characterized, and quantitated on a single LC/MS/MS system. The high sensitivity full scan MS and MS/MS spectra allows the identification of more metabolites, and the comprehensive Metabolite ID application software automates the process. Identify more metabolites with more confidence with the Q TRAP LC/MS/MS system.

Figure 5. TIC of a neutral loss scan of 176 identifies three glucuronide metabolites of buspirone. MS/MS and MS3 of hydroxy-buspirone glucuronide.

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