

Strategies for Simultaneous Determination of Nature and Extent of Metabolism Using a Linear Ion Trap Mass Spectrometer

Authors: Gargi Choudhary, Diane Cho presented by: Paul Humphrey

Thermo Electron, San Jose,

Overview

Purpose: To demonstrate a strategy for rapid determination of the nature and extent of metabolism

Methods: LC-MSⁿ with electrospray ionization was used for rapid analysis of samples using the Finnigan™ LTQ™ linear ion trap mass spectrometer

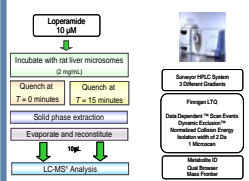
Results: Simultaneous metabolite identification and determination of metabolic stability in a single chromatographic run is possible due to the ultra-fast cycle time and high MSⁿ sensitivity of the Finnigan LTQ.

Introduction

Absorption, distribution, metabolism and excretion (ADME) studies make up key components in the drug discovery process. Recently, metabolite characterization has become one of the main drivers in the drug discovery process, helping to optimize ADME properties and increase the success rate for drug development. A high throughput strategy to accelerate the lead optimization phase in the drug development cycle will be presented in this poster. This strategy involves the simultaneous determination of the nature and extent of metabolism by performing metabolite identification and metabolic stability measurements in a single analytical run.

Experimental

FIGURE 1. Experimental Workflow



Eight drugs with low, medium and fast metabolic rates were used for simultaneous stability and the metabolite identification study. The experiments were performed using a Finnigan LTQ linear ion trap mass spectrometer according to the workflow illustrated in Figure 1. LC run times for the three different gradients were: 2 min., 4 min., and 8 min.

An automated five scan event LC-MSⁿ method (Figure 2) including MS, MS/MS and MS³ scans with Data Dependent™ acquisition was utilized. The full scan MS data points were used to determine metabolic stability, whereas the MS/MS and MS³ scan data were used for structural characterization of the metabolites. The mass spectrometer cycle time for the five scan event acquisition method is less than 1200 ms.

FIGURE 2. Automated Data Dependent Acquisition for Five Scan Event LC-MSⁿ Analysis

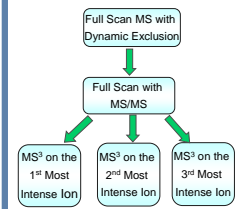
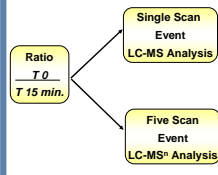


FIGURE 3. Comparison of Metabolic Stability Determination using the Single and Five Scan Event Methods



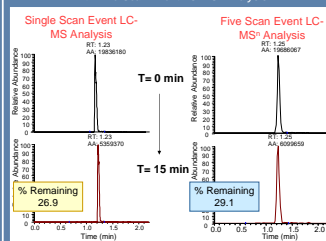
Metabolic stability data obtained from the five scan event LC-MSⁿ method (Fig. 3) was compared against that obtained from the single scan event method.

Results

Traditionally, metabolic stability and metabolite profiling experiments are carried out as two separate assays. Metabolic stability is determined from an LC-MS analysis in SIM or SRM mode whereas metabolite identification and characterization requires an LC-MSⁿ experiment. Kantharaj et al (1,2) have proposed that metabolic stability and metabolite profiling experiments be performed simultaneously in a single data dependent LC-MSⁿ analysis. In this poster, we demonstrate this strategy for increasing throughput in the lead optimization phase using the Finnigan LTQ linear ion trap mass spectrometer. Metabolic stability, or the percentage of drug remaining, was determined from the peak area ratios obtained at time 15 and 0 minutes after sample incubation. The peak areas are defined by the full scan MS data points acquired using the single and five scan event methods.

FIGURE 4. Determination of Metabolic Stability for Loperamide

Comparison of Single Scan Event LC-MS Analysis with Five Scan Event LC-MSⁿ Analysis



The metabolic stability for loperamide, a drug with medium rate of metabolism, shown in Figure 4 was obtained from a run using ultra-fast chromatographic conditions (2 min. total LC run time). The value determined from single scan event LC-MS analysis is in good agreement with that determined from the five scan event LC-MSⁿ method (26.9% vs 29.1%, respectively).

FIGURE 5. Determination of Metabolic Stability for Buspirone

Comparison of Single Scan Event LC-MS Analysis with Five Scan Event LC-MSⁿ Analysis

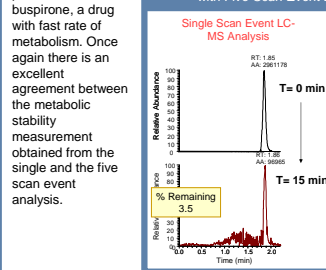


Figure 5 shows results from a similar experiment performed for buspirone, a drug with fast rate of metabolism. Once again there is an excellent agreement between the metabolic stability measurement obtained from the single and the five scan event analysis.

FIGURE 6. Determination of Metabolic Stability for Eight Drugs with Slow, Medium and Fast Rate of Metabolism

Drug	% Remaining	
	Single Scan event LC-MS Analysis	Five Scan Event LC-MS ⁿ Analysis
Warfarin	89.2	84.9
Loperamide	26.9	27.5
Diclofenac	12.6	14.2
Bifonazole	14.5	14.9
Imipramine	2.6	2.7
Buspirone	3.5	3.5
Midazolam	<1	<1
Nicardipine	<1	<1

Results in Figure 6 show that the five scan event method is indeed valid for measurement of metabolic stability for drugs with slow, (warfarin), medium (loperamide) and fast (diclofenac, bifonazole, imipramine, buspirone, midazolam and nicardipine) rate of metabolism.

In the same analytical run, we derive qualitative information for the identification metabolites from the MS/MS and MS³ scan events. Figure 7 illustrates the characterization of metabolites of loperamide using ultra-fast chromatographic conditions. The base peak chromatogram suggests several co-eluting metabolites are present in the 15 minute incubation sample. These metabolites were identified using Metabolite ID software. The extracted ion chromatograms are illustrated in Figure 8. In addition to the three major metabolites M1 (m/z 479), M2 (m/z 493) and M3 (m/z 463), two additional metabolites M6 (m/z 471) and M7 (m/z 441) are also identified. These (M6 and M7) are rat-specific metabolites.

FIGURE 7. Rapid Characterization of Metabolites with Ultra-Fast Chromatographic Conditions using the Five Scan LC-MSⁿ Method

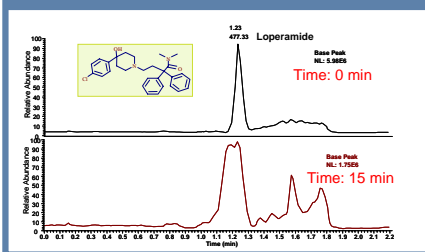
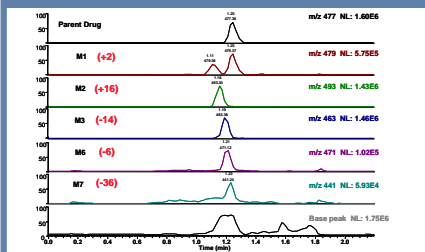


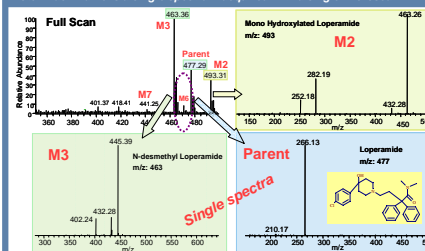
FIGURE 8. Extracted Ion Chromatograms for Metabolites of Loperamide Identified using Metabolite ID



In Figure 9, the full scan MS spectrum shows ions from multiple co-eluting components including the parent drug (m/z 477). MS/MS spectra obtained on three of the co-eluting components are also shown. This illustrates the ability to improve throughput for metabolite identification with the use of ultra-fast LC gradients, even in the presence of co-eluting compounds. This is feasible because of the ultra-fast cycle time and high spectral quality of the Finnigan LTQ linear ion trap mass spectrometer.

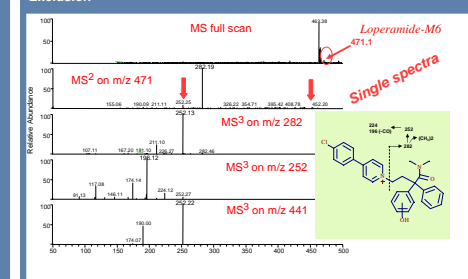
FIGURE 9. Full Scan MS and MS/MS Spectra of Loperamide and Co-eluting Metabolites

Note: Each frame is a single spectrum acquired with a single microscan



Automated Data Dependent acquisition with Dynamic Exclusion™ allows for further structural information to be obtained on low intensity ions as illustrated in Figure 10. The MS/MS spectrum of m/z 471 (<10% abundance in the MS spectrum), is shown, together with a single MS³ spectrum for each of the three most intense MS/MS ions, demonstrating the high MSⁿ spectral quality generated on the Finnigan LTQ at ultra-fast cycle time.

FIGURE 10. Automated Data Dependent Analysis with Dynamic Exclusion



Conclusions

Strategies and techniques presented in this poster can be applied to accelerate the drug development process. The ultra-fast cycle time and ultra-high sensitivity MSⁿ performance of the Finnigan LTQ were utilized for simultaneous determination of metabolic stability and metabolite identification thereby achieving high throughput screening of drug candidates. The ability to resolve co-eluters while maintaining high spectral quality enables the use of ultra-fast LC gradients resulting in >400% increased analytical throughput. Automated Data Dependent acquisition with Dynamic Exclusion presents a powerful capability for the acquisition of MS/MS and MS³ spectra on low intensity ions.

References

- 1) Kantharaj et al., *Rapid Commun. Mass Spectrom.* 2003, 17, 2661-8
- 2) Kantharaj et al., *Rapid Commun. Mass Spectrom.* 2005, 19, 1069-74

Acknowledgement

The authors would like to thank Ji Ma, Robert Cho and Shichang Miao from Amgen-San Francisco for help with sample preparation and technical discussions.

All trademarks are property of Thermo Electron Corporation.

Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION