Evaluation of Drug Microsomal Stability Using Ultra-High Throughput Laser Diode Thermal Desorption Combined With a High Resolution Mass Spectrometer

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Overview

Purpose: To evaluate a high throughput sample introduction technology on a benchtop high resolution mass spectrometer for eADME applications.

Methods: For LDTD analysis, sample dilution (1:20 in 75% methanol/water) was performed and 3 µL of solution was deposited onto a Laz-well™ sample plate for analysis via laser desorption on a benchtop quadrupole Orbitrap mass spectrometer. The percent remaining of each sample at each time point was calculated and the half-life of each compound was estimated using Thermo Scientific QuickCalc data review software.

Results: Analysis time per sample improved by 7.5 fold when compared to a conventional LC method (20 seconds per sample compared to 3 minutes). Utilizing a generic HR/AM detection method removed the need for MS method development.

Introduction

Pharmaceutical laboratories performing high throughput screening assays are constantly evaluating new technologies to increase throughput. Improvements in sample introduction and detection techniques can shorten analytical methods from minutes to seconds. The Laser Diode Thermal Desorption (LDTD™ Phytronix Technologies, Quebec, Canada) ion source is a novel technology that accommodates ultra-high throughput sample analysis. Unlike LC-MS, LDTD does not utilize organic mobile phase and liquid chromatography, but instead uses a laser diode to indirectly irradiate samples for introduction. Coupling this rapid introduction technology to a MS system that can operate in a generic analytical fashion can efficiently improve throughput for discovery applications. In this study, we evaluated the potential LDTD to effectively perform microsomal stability studies on a high-resolution accurate mass MS detector.

Methods

Sample Preparation

Six commercially available compounds were incubated at 37 °C in human and rat liver microsomes at 3µM and 300nM starting concentrations in a 100 mM NaPO4, pH 7.4, 5 mM $MgCl_2$ buffer solution. The incubation was quenched at several time points between 0 and 45 minutes using Methanol: Acetonitrile (1:3, v:v) followed by centrifugation. After quenching the reaction, supernatants were split for analysis by LC and LDTD HRMS on a Thermo Scientific Q Exactive benchtop quadrupole Orbitrap mass spectrometer. LC analysis was performed on supernatants directly for HRMS analysis on an identical mass spectrometer using heated electrospray. The percent remaining of each sample at each time point was calculated and the half-life of each compound was estimated.

Sample Introduction

For LDTD analysis, further sample dilution (1:20 in 75% methanol/water) was performed and 3 µL of solution was deposited onto a sample plate for analysis. The sample was dried under nitrogen for 5 minutes. Laser desorption was performed using a generic pattern for all samples, where the laser was ramped to 45% power over 5 seconds and held for 2 seconds before returning to 0%. This ramp is required to create a peak shape for the analyte detection process.

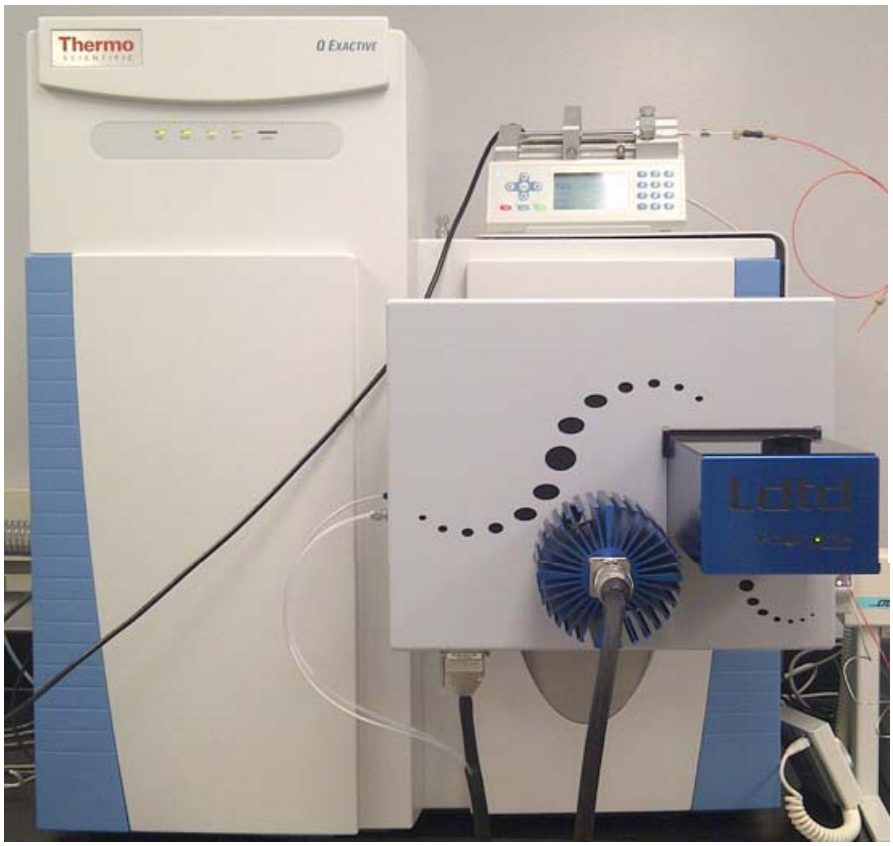
Mass Spectrometry

Samples were analyzed on a Q Exactive™ bench-top quadrupole Orbitrap mass spectrometer in positive polarity mode. All data was collected with a generic method suitable for the mass range of the compounds selected. The method was set to scan from 200-600 *m/z*, and all data was collected at 35,000 resolution.

Data Analysis

Data was acquired using Thermo Scientific Xcalibur 2.2 software. For peak integrations and half-life calculations, data was processed, reviewed and reported using QuickCalc/GMSU™ software (Gubbs Inc, Alpharetta, GA).

FIGURE 1. LDTD ion source attached to the Q Exactive benchtop quadrupole Orbitrap Mass Spectrometer. Conversion to a standard LC and autosampler can be performed in minutes.

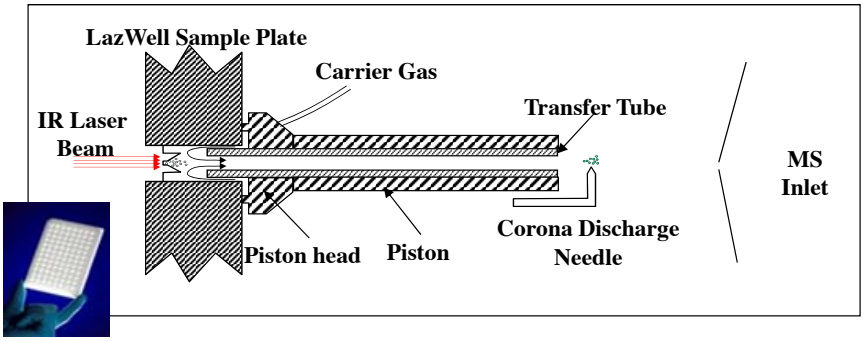


Results

The metabolic stability data generated from LDTD-HRMS appeared in line with expectations. All LDTD results were obtained with the same generic laser desorption and full scan HRMS method, eliminating the need for individual compound method development. LDTD was shown to be a suitable technique for stable compounds (minapraine) as well as compounds with high rates of metabolism (buspirone.) Total sample-to-sample time by LDTD was approximately 20 seconds, allotting 8 seconds for software cycle time between injections. To improve throughput, samples were additionally tested in a single file injection format, reducing the sample-to-sample time to 12 seconds. Since detection was performed in full scan across a range of 200-600 amu, the data obtained could be mined for phase 1 metabolites expected from the incubation (for example hydroxy and dihydroxy metabolites of buspirone). All components monitored were within 5 ppm of expected results throughout the study. Additionally, the six compounds were pooled to improve throughput and showed identical results to individually studied samples.

It was found that the ionization of certain types of compounds the response were different in pure form and in the biological matrix when using LDTD, causing a potential reduction in sensitivity due to the presence of residual of matrix. The additional dilution of the samples in 75% methanol/water improved the sensitivity for analysis.

FIGURE 2. LDTD Operational principals and schematic. LDTD sample plate is shown in the lower left corner.



- Samples are dried in standard 96-well plate with a metal sheet insertion
- Thermal desorption induced by a laser at 980 nm (no photon-sample interactions)
- Gaseous neutral species transferred by a carrier gas
- Ionization occurs into the corona discharge region

FIGURE 3. QuickCalc/GMSU data processing software. Sample inclusion/exclusion or integration modifications are immediately reflected in the calculated results table for half life.

Data Processing and Review:

All results were reviewed and tabulated using QuickCalc/GMSU software. Data is organized by compound (top), and each injection series was reviewed in the Pseudo HT Acquisition Display (middle) to rapidly check integrations and visually inspect the trend of metabolism. With appropriate constants entered into a time point set applied in the software, calculations for half life were obtained (right). Additionally with these constants, calculations for intrinsic hepatic clearance and predicted hepatic clearance could be calculated.

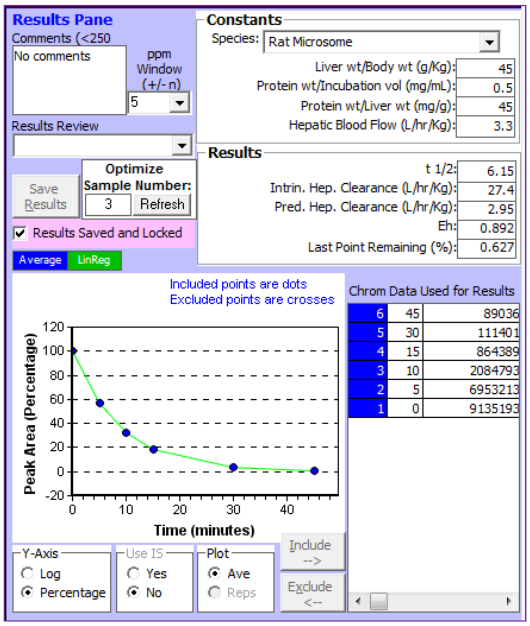


Table 1. Calculated results for t ½ of incubations

Analyte Name	Species Experiment	Half Life (Minutes)	% Last Pt Rem
Buspirone (H)	Human Microsome	16.00	14.30
Buspirone	Rat Microsome	6.15	0.63
Diltiazem (H)	Human Microsome	40.30	46.10
Diltiazem	Rat Microsome	2.66	0.00
Minaprine (H)	Human Microsome	33.00	38.80
Minaprine	Rat Microsome	18.60	18.70
Mirtazapine (H)	Human Microsome	70.70	64.30
Mirtazapine	Rat Microsome	16.80	15.50
Omeprazole (H)	Human Microsome	47.10	51.60
Omeprazole	Rat Microsome	26.30	30.60
Verapamil(H)	Human Microsome	22.80	25.50
Verapamil	Rat Microsome	13.30	9.61

Pooled sample results

To improve throughput of analysis by LDTD, samples for the incubations were pooled by time point. Using the same dilution scheme for individual injections, the rat microsome samples were pooled together by time point and 3uL of each pooled sample were spotted onto the LDTD plate. This technique allowed for all 6 compounds to be analyzed consecutively. Results for the pooled sample analysis corresponded well to the individual injections (Table 2) and allowed for a 6-fold increase in throughput. With the sample dilution, all components were still detected well enough to calculate t ½ values (Diltiazem was not detected at t30, and t45 due to dilution and rapid turnover in the incubation)

Table 2. Comparison of t ½ of incubations for rat microsomes- individual sample injections versus pooled sample injections

Analyte Name	Species Experiment	Half Life-Individual (Minutes)	Half Life-Pooled (Minutes)
Buspirone	Rat Microsome	6.15	5.93
Diltiazem	Rat Microsome	2.66	3.24
Minaprine	Rat Microsome	18.60	23.20
Mirtazapine	Rat Microsome	16.80	19.60
Omeprazole	Rat Microsome	26.30	23.20
Verapamil	Rat Microsome	13.30	16.20

Figure 4. Comparison of LDTD acquisition sample time (top) versus LC run (3 minutes- bottom)

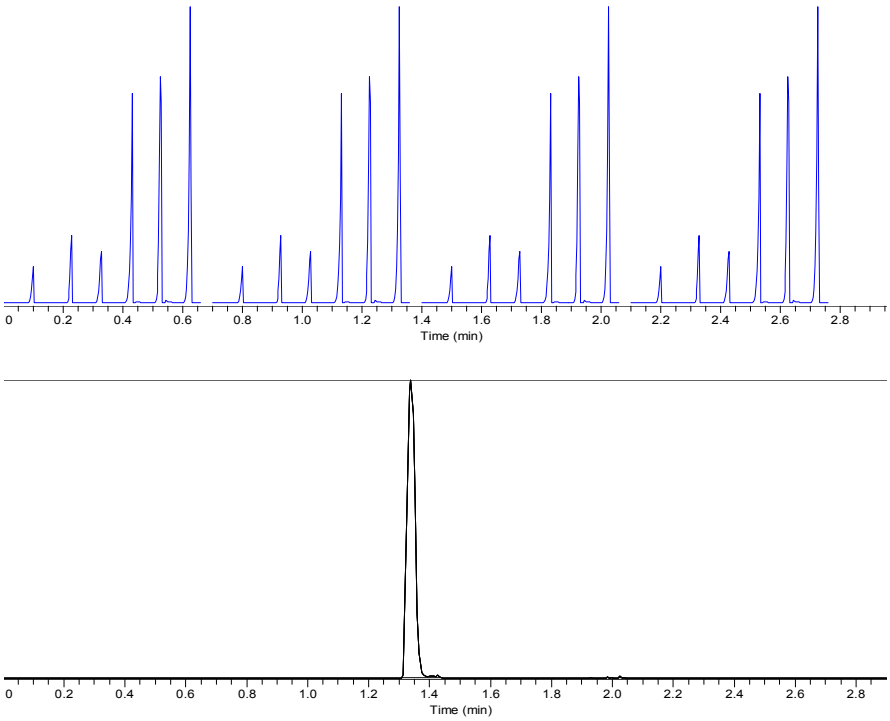
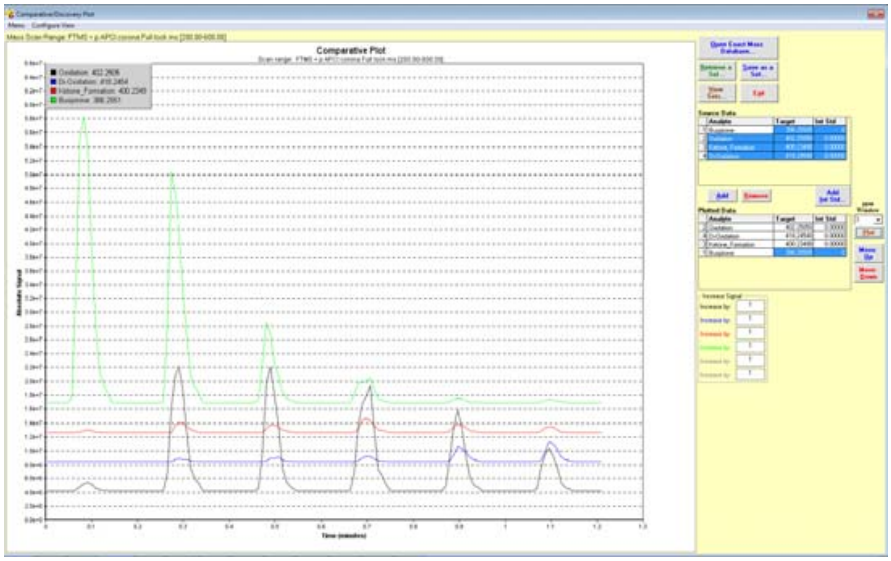


Figure 5: QuickCalc Comparative/Discovery Plot of Buspirone rat incubation samples (0-45 minutes) plotting four common phase 1 modifications (3 ppm mass window)



Phase 1 Metabolism

Since a generic, full scan acquisition method was employed on the Q Exactive benchtop quadrupole Orbitrap mass spectrometer, the metabolic stability samples could additionally be mined for common modifications. In the example shown in Figure 5, three common phase 1 metabolites for buspirone were investigated and a trend of formation/disappearance could be observed. Since LDTD is not a separation based sample introduction, it is not possible to discern between different species of the same metabolite (for example there are multiple species of buspirone oxidation).

Conclusion

- LDTD HRMS proved to be a suitable analytical technique for metabolic stability applications and allows for improved throughput when compared to typical LC analysis.
- Pooling samples allowed for a 6-fold increase in analysis on the LDTD with comparable results.
- Employing a generic HRAM method eliminated the need for MS method development and also allowed for additional data re-interrogation for phase 1 metabolism.

Acknowledgements

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References

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