

# **MULTI-DIMENSIONAL BALLISTIC HPLC ESI-TOF/MS FOR HIGH THROUGHPUT LEAD OPTIMIZATION IN DRUG DISCOVERY**

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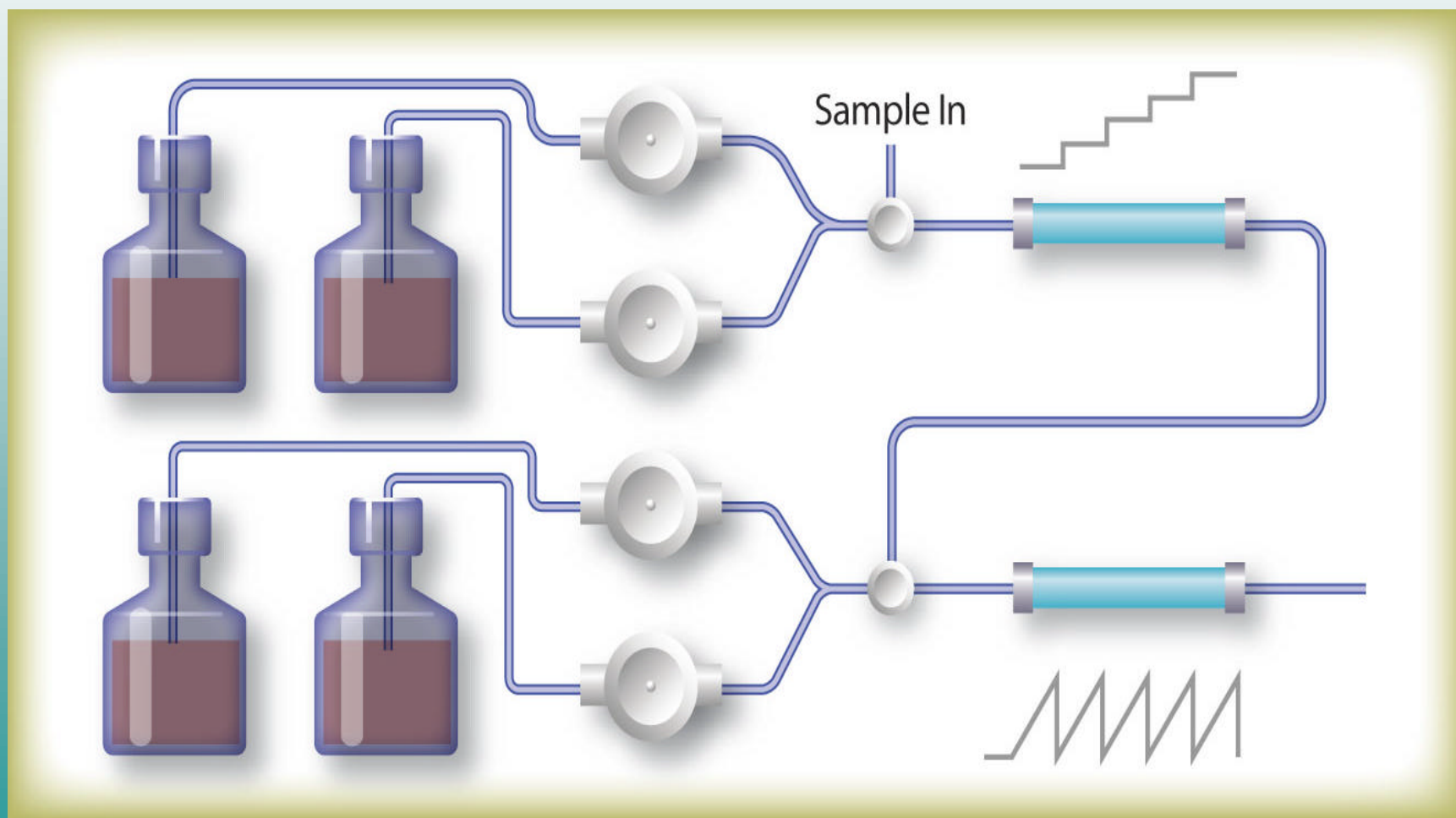
# INTRODUCTION

Over the past decade great strides have been made in the synthesis and screening of large libraries of small molecules. Companies that used to produce a few potential drug candidates a year are now producing hundreds to thousands of lead candidates each year. These lead candidates must be quickly evaluated for a variety of pharmaceutical characteristics (PC) to insure that only those with the highest probability for success are taken into drug development. Ballistic Multi-Dimensional Liquid Chromatography (MDLC) coupled with ESI-TOF/MS is a powerful tool for quickly gathering the data necessary to optimize this phase of the drug discovery process. Although the value of early information on the PC of lead candidates is recognized in the industry, some standardization in methodology and data handling will be required to make this a manageable task. This study introduces a global MDLC/MSD platform and methodologies to help with the analytical portion of this task. Using this platform, 384 to 1536 samples can be analyzed in a single day.

# EXPERIMENTAL

- **HT Autosampler**      **Michrom Paradigm MX4**  
**4 x 96 or 4 x 384 Microtiter Plates**
  
- **Ballistic MDLC**      **Michrom Paradigm MX4**  
**4 x High Pressure Pumps**  
**2 x 10 Port Valves**
  
- **Detection**      **Michrom Paradigm MX4**  
**UV/VIS**  
**ESI-TOF MS**

# BALLISTIC MULTIDIMENSIONAL LC ON MX4



# BALLISTIC MDLC TOF/MS FOR PC ASSAYS

The new Michrom Paradigm MX4 LC/MSD is an integrated LC/MSD system that combines a High Throughput Autosampler (CTC Mini Pal), a Ballistic Multi-Dimensional Separations Module (Michrom Paradigm MS4) and an ESI-TOF Mass Selective Detector (Michrom) with applications kits and software developed for a variety of pharmaceutical and biochemical applications.

The MX4 is well suited for the high throughput needs of analytical chemists trying to determine the pharmaceutical characteristics (PC) of a wide variety of compounds for drug lead optimization. The autosampler is capable of running 4x96 or 4x384 samples with a 15 second inject to inject cycle, including wash. The MDSM is capable of running 1D to 4D LC with automated column switching and ballistic gradients, which allows simple PC assays to be run in 0.2 - 1 minutes and more complex PC assays to be run in 2-10 minutes. The ESI-TOF MSD provides the sensitivity, selectivity, resolution, mass accuracy and fast data sampling and storage (10 Hz) needed for these high throughput PC applications.

# PHARMACEUTICAL CHARACTERISTICS

## Simple LC/MSD Methods

- **Physical Stability**
- **Lipophilicity**
- **Solubility**
- **Salt Form**
- **pKa**
- **Adsorption**

## Complex LC/MSD Methods

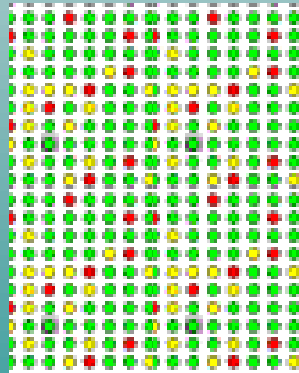
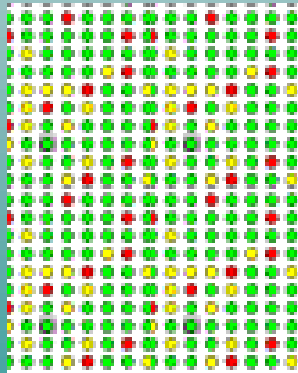
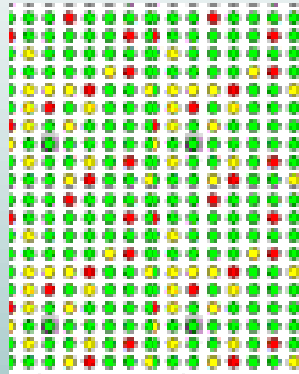
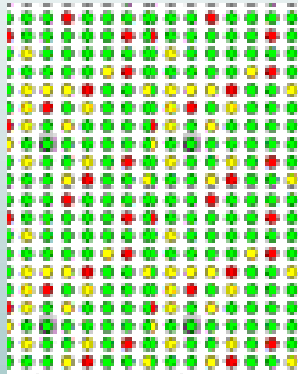
- **Metabolic Stability**
- **Pharmacokinetics**
- **Excretion**
- **Toxicity**
- **Bioaffinity**
- **Biomarkers**

# 1D BALLISTIC HPLC FOR SIMPLE ASSAYS

Many of the pharmaceutical characteristics (PC) that need to be accessed during lead optimization require very simple tests that would only require a basic LC/MS protocol. For these simple assays, the MX4 was set up in a 1D ballistic column switching mode, which provides fast gradients (0.2-1.0 minutes) and allows re-equilibration of one column and preloading of the next sample while the second column is running the current assay.

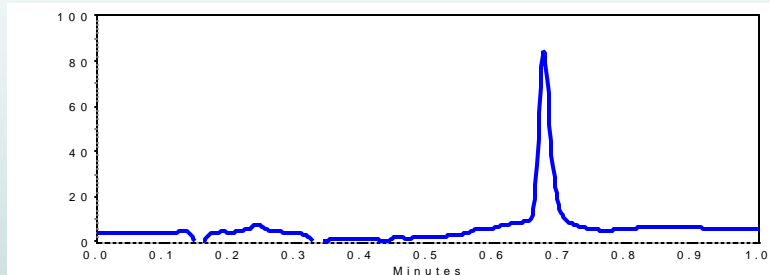
The example on the following slides shows the analysis of the physical stability (in water at 25°C) for 1536 lead candidates (4 x 384) in a single day (1 minute total analysis time per sample). The MX4 displays the data in a standard plate format, with colors based on user defined criteria. If specific data (UV, TIC, XIC, BPT, Mass Spectra) for individual samples is required, a simple click on the sample well of interest can bring up the information specific to that sample.

# DISPLAY OF 1536 STABILITY ASSAYS

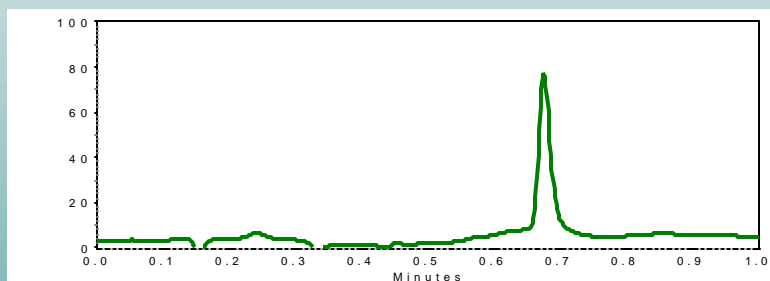


- Green wells represent lead candidates that showed good physical stability
- Yellow wells represent lead candidates that showed problems with the physical stability assay
- Red wells represent lead candidates that showed poor physical stability

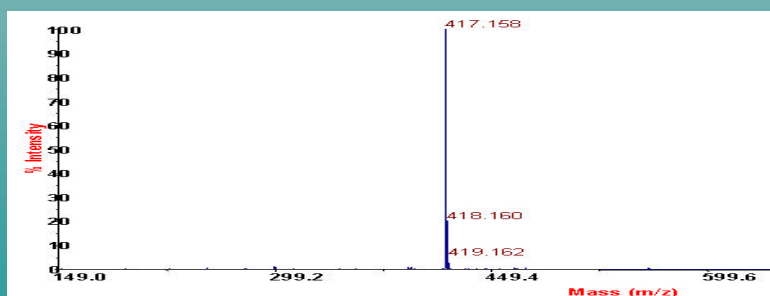
# LC/MSD OF STABLE LEAD CANDIDATE



- Upper Trace Shows XIC (m/z 417.2) of Plate 1 A1 Standard in Water

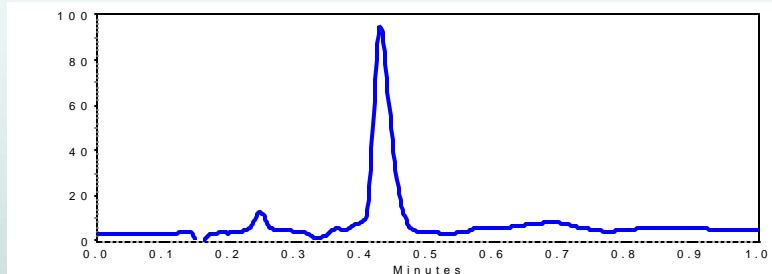


- Lower Trace Shows XIC (m/z 417.2) of Plate 1 A1 in Water After 24 Hours

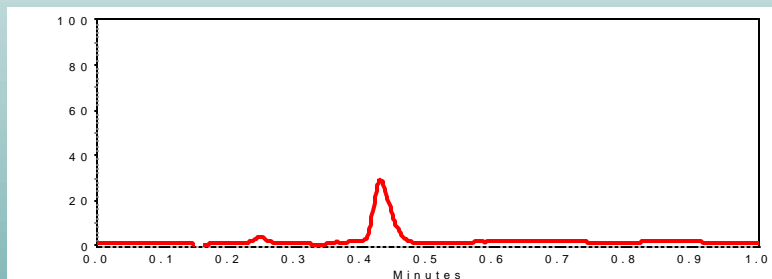


- Mass Spectra for Plate 1 A1 at RT 0.683 minutes

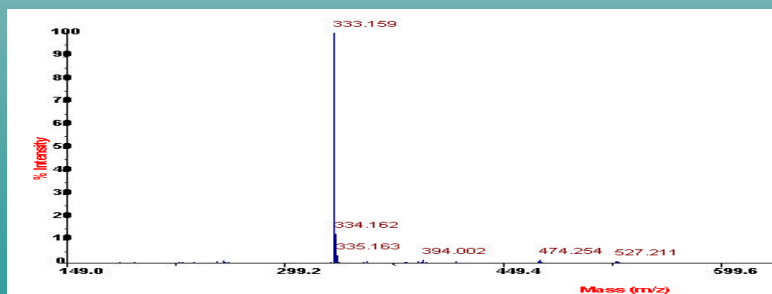
# LC/MSD OF UNSTABLE LEAD CANDIDATE



- Upper Trace Shows XIC (m/z 333.2) of Plate 1 C1 Standard in Water



- Lower Trace Shows XIC (m/z 333.2) of Plate 1 C1 After 24 Hours



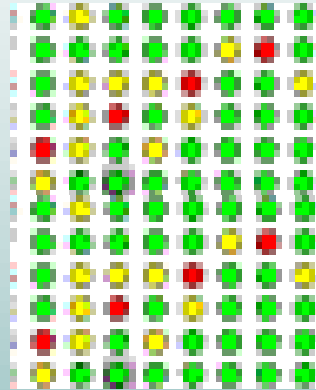
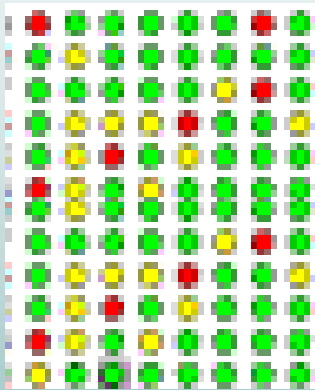
- Mass Spectra for Plate 1 C1 at RT 0.43 minutes

# MD BALLISTIC HPLC FOR COMPLEX ASSAYS

Several pharmaceutical characteristics (PC) that need to be accessed during lead optimization require more advanced protocols. For these complex assays, the MX4 can be set up in high throughput, multidimensional column switching modes, which provides fast sample purification (2-10 minutes) coupled with fast gradient analysis (1.0-5.0 minutes) with UV and MS detection.

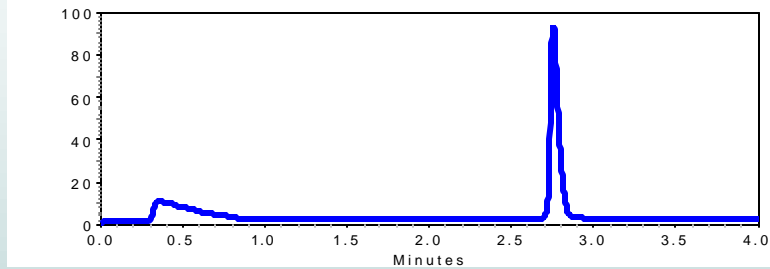
The example on the following slides show the analysis of the metabolic stability (liver microsomes) for 384 lead candidates (4 x 96) in a single day (5 minute total analysis time per sample). The MX4 displays the data in a standard plate format, with colors based on user defined criteria. If specific data (UV, TIC, XIC, BPT, Mass Spectra) for individual samples is required, a simple click on the sample well of interest can bring up the information specific to that sample.

# DISPLAY OF 384 METABOLISM ASSAYS

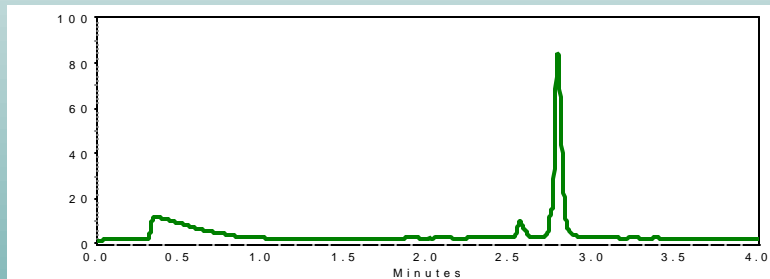


- Green wells represent lead candidates that showed good metabolic stability
- Yellow wells represent lead candidates that showed problems with metabolic stability
- Red wells represent lead candidates that showed poor metabolic stability

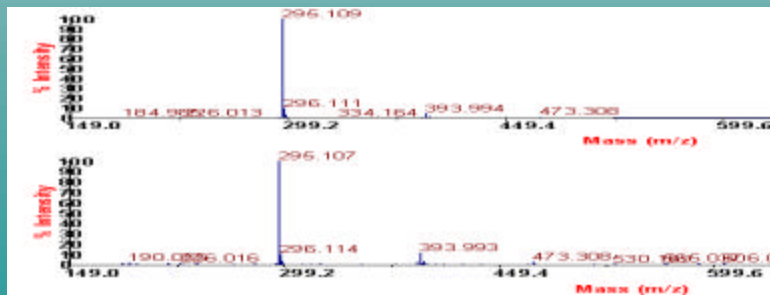
# LC/MSD OF LEAD WITH GOOD METABOLISM



- Upper Trace Shows TIC Plate 2 A1 Time Zero

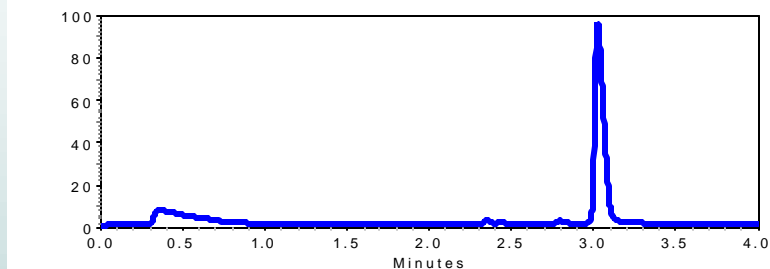


- Lower Trace Shows TIC of Plate 2 A1 After 24 Hours

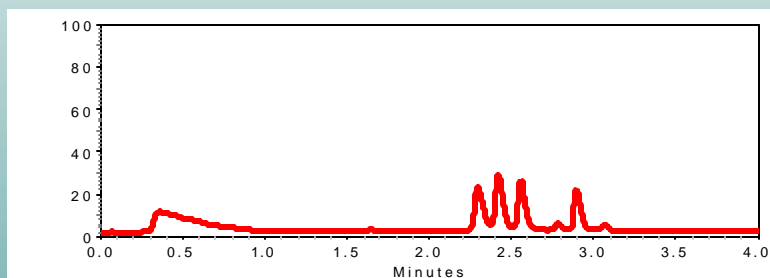


- Mass Spectra for Plate 2 A1 at RT 2.76 minutes  
Upper Spectra = Time 0  
Lower Spectra = Time 24

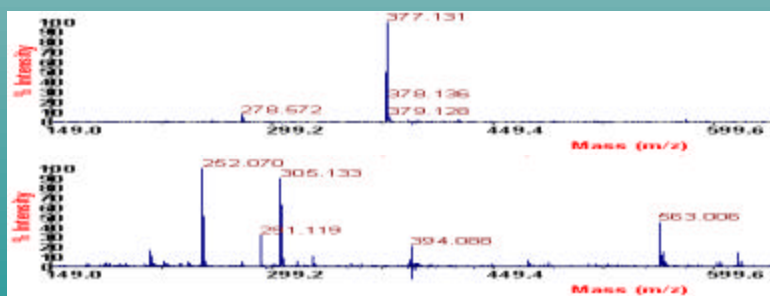
# BALLISTIC LC/MSD OF POOR METABOLISM



- Upper Trace Shows TIC Plate 3 A1 Time Zero



- Lower Trace Shows TIC of Plate 3 A1 After 24 Hours



- Mass Spectra for Plate 2 A1 at RT 3.08 minutes  
Upper Spectra = Time 0  
Lower Spectra = Time 24

# CONCLUSIONS

- **The Paradigm MX4 is an Ideal Workstation For PC Screening**
- **Ballistic MDLC With ESI-TOF MS Provides High Throughput**
- **Speed, Resolution and Sensitivity Optimized by Application**
- **Application Specific Kits Provide Standardization For PCs**
- **Software Customized For Specific PCs and User Needs**