What is a Mass Spectrometer

Mass Spectrometers measure:
- atomic weight of single elements (ICP-MS)
- the molecular weight of molecules (LCMS, MALDI MS)
Newborn Screening for metabolic conditions by LC-MS/MS

- **Newborn screening** is the process of testing newborn babies for treatable diseases:
  - genetic
  - endocrinologic
  - metabolic
  - hematologic

Newborn Screening Branch
Genetic Disease Screening Program
www.dhs.ca.gov/nbs
Neonatal Screening Blood Specimen Collection:

- A few drops of blood taken from the baby’s heel are put on special filter paper. The blood is then sent to a state-approved lab for testing.
Metabolic Diseases

- Metabolic diseases affect the body’s ability to use certain parts of food for growth, energy, and repair.

- The parts include:
  - Amino acids from proteins,
  - Fatty acids from fats
  - Organic acids from proteins, fats, and sugars.
Some of the metabolic diseases screened for by the NBS Program:

- Phenylketonuria (PKU)
- Galactosemia
- Maple Syrup Urine Disease (MSUD)
- Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)
- Biotinidase Deficiency
25-Hydroxy Vitamin D by LC/MS/MS

- Vitamin D is clinically useful to diagnose disorders related to intestinal malabsorption and vitamin D deficiency or intoxication.
- The 25-Hydroxy vitamin D blood test allows monitoring for therapeutic response in patients being treated for vitamin D-related disorders.
Background

- 25-Hydroxy vitamin D (250HD) is the major circulating form of vitamin D and the precursor of the active form (1,25-dihydroxy vitamin D). It has a long half-life, which makes its measurements ideal for assessing a patient’s vitamin D level.
Components of a Mass Spectrometer

**Source**
- Form ions (charged molecules)
- **Inlet**
  - Solid
  - Liquid
  - Vapor
  - Method to vaporize sample

**Mass Sorting**
- Sort Ions by Weight

**Detection**
- Detect Ions
- Ion Detection

**Data Analysis**
- Graph indicating mass spectra
LCMSMS – What can it do?

- The two most important uses of LC/MS/MS
  - Identify unknown species (structural elucidation)
  - Quantification (how much of an analyte is in your sample)

- Technologies
  - Spherical and Linear Ion Traps
  - Time-of-Flight (TOF) MS & Hybrid Quadrupole TOF MS
  - Quadrupole based MS
  - Hybrid Quadrupole – Linear Ion Trap MS

- Application areas
  - Small molecules
  - Proteomics and Biomarker research
Benefits of LCMSMS

- Extreme detection power for single and multi analyte analysis
- Often simplified sample preparation (typically no derivatization needed)
- Can be used to identify unknowns (structural elucidation) and quantitation
- Simpler LC methods and shorter LC run times >> more throughput
- Analytes from low to very high polarity can be efficiently ionized
- Rugged instrument design and high throughput
  - Lower cost/sample
  - Increased productivity
  - Easy-to-use
Instrument performance criteria which matter

- Ion source & interface design
- Number of analytes that can be quantified from single injections
- Minimum dwell time and pause time and consequences on Signal/noise
- Absence of cross-talk
- Detector technology
- Sensitivity, signal/noise (S/N) & detection power
- Dynamic range
- Scan speed
- Systems ruggedness when analyzing many real samples
System Components

- **Eluent Reservoir**: Houses the mobile phase continuously applied to the column. Usually consists of two bottles, one aqueous and one organic.

- **Pump**: Accurately delivers eluent to the column. Capable of forming precise eluent gradients.

- **Guard Column**
  - **Thermostatted Column Compartment**: Stable temperature ensures constant retention times and may reduce column backpressure.

- **Analytical Column**: The heart of an HPLC system. The column separates the individual analytes from the matrix.

- **Sample Introduction**: Introduces the sample into the mobile phase stream.

- **Detection**
  - **Refractive Index**
  - **ELSD**
  - **Mass Spectrometer**: The detector monitors the presence of a compound in the mixture.

- **Data Acquisition and Instrument Control**: Interprets the data collected from the detector.
Reversed Phase Mechanism

1. The autosampler picks up sample from the vial.

2. The sample is injected onto the column.

3. Initially, the pump delivers a low concentration of organic solvent, and the analytes “stick” on the column.

4. As the concentration of organic solvent increases, the analyte “unsticks” from the column, separating one analyte from another.
Reversed Phase Mechanism

Aqueous Mobile Phase

silica ( -OH)

H₂O  H₂O

H₂O  H₂O

H₂O  H₂O

H₂O  H₂O

H₂O  H₂O

H₂O  H₂O
Reversed Phase Mechanism

Acetonitrile in Mobile Phase

silica ( -OH)
HPLC versus UHPLC

- Conventionally, HPLC analysis is run on columns with a particle size 3µm or larger
  - Useful for samples with high matrix
  - Works with standard HPLC pumps

- Recently, there has been a movement to columns packed with column particles ≤2µm
  - Less diffusion in the column
  - Decreases the chromatographic run time
  - Produces sharper peaks
  - Requires pump capable of increased column backpressure

- Both methods have advantages, and each is easily coupled to a mass spectrometer
How Mass Spectrometry Works – The Basics

1. Ionized compounds are introduced into the instrument

2. Ions are sorted by m/z

3. The signal is detected and counted

4. The results are displayed
Convenient “Plug and Play” Sources

**Turbo V™** source with ESI & APCI probes
- Efficiently ionizes compounds and eliminates cross contamination
- Accommodates large sample loads and LC flow rates up to 3ml/min
- Embedded ceramic heater technology and improved gas dynamics enable low detection limits
- Quick change TurbolonSpray® probe and APCI probe let you switch ionization modes in seconds

Optional **DuoSpray™** ion source
Optional **PhotoSpray®** source
Electrospray Ionization Theory (positive mode shown)

1. Formation of Charged Droplets
2. Evaporation
3. Ionic repulsion w/clustering
4. Ions enter Mass Analyzer

Ion Spray Voltage (IS)
LC Flow
Gas 1 (GS1)
Gas 2 (GS2)
& Heat (TEM)
Curtain Plate
Curtain Gas (CUR)
Orifice (DP)

- Green: Droplet
- Yellow: Solvent molecule
- Blue: Ion of interest
APCI Theory

1. Molecules in gas phase
2. Corona discharge needle ionizes N2 or O2 in source
3. N2 or O2 pass charge to vaporized solvent
4. Vaporized, charged solvent passes charge to Analyte
5. Ions enter Mass Analyzer aided by DP

- O2 or N2
- Solvent molecule
- Ion of interest
When to use the various sources

- **TurbolonSpray**: High polarity, high molecular weight (100kD), suitable for proteins.
- **Photospray**: Medium polarity, medium molecular weight (10kD), suitable for peptides.
- **APCI**: Medium polarity, low molecular weight (1kD), suitable for carbamates, steroids, PAH's.
Triple Quadrupole Overview

1. Ion Production
2. Ion Transmission
3. Mass Filter
4. Fragmentation
5. Mass Filter
6. Detector
Triple Quadrupole (Tandem) MS schematics
LC/MS/MS – Multiple Reaction Monitoring (MRM)

- Superior selectivity and sensitivity for quantitation of targeted compounds
- MRM ratio calculation for compound identification (qualifier/quantifier)
- Multi-target screening for hundreds of compounds
Multiple Reaction Monitoring (MRM)

Scan type: MRM (MRM)

Polarity
- Positive
- Negative

Product Of: 232.000 (Da)

<table>
<thead>
<tr>
<th>Q1 Mass (Da)</th>
<th>Q3 Mass (Da)</th>
<th>Time (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>232</td>
<td>132</td>
<td>100</td>
</tr>
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N$_2$

Q0  Q1  Q2  Q3  Detector

MRM

LC

intensity

0  5 min

time

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Single Quadrupole vs. Triple Quadrupole

- Higher selectivity resulting in better S/N for quantitation
- Better accuracy and reproducibility
- Wider linear range
- More reliable identification using MRM ratios
How quantitation is performed

Run calibration curve

Integrate curve

Integrate sample
Confirmation through MRM

- LCMSMS analysis usually requires monitoring 2 MRM transitions for each compound – Quantitation Ion and Qualifier Ion
- Ion ratio (ratio of the areas of the 2 transitions) needs to be calculated

- Oxazepam
  - Quant 287>241
  - Qualifier 287>269

- Nordiazepam
  - 271>140
  - 271>165

- Lorazepam
  - Quant 321>275
  - Qualifier 321>229

- Temazepam
  - 301>177
  - 301>193
Scheduled MRM™ Algorithm

- Adjusts detection windows automatically depending on retention time
- Allows detecting many more MRM transitions
- Allows using faster LC
QTRAP® System Overview

• Q3 can be used as a quadrupole or as a linear ion trap
Q1 MS Scan vs. Enhanced MS Scan
Product Ion Scan vs. Enhanced Product Ion Scan

Product Ion Scan

Enhanced Product Ion Scan
Movie

..\Help\Brent\4000QTRAP_4x3_640x480_052808.wmv
Information Dependent Acquisition (IDA)

- Acquisition method that automatically selects candidate ions for MS/MS study

Wide variety of survey or independent scans possible

Selection criteria

Dependant scan (EPI, Product Ion and MS3)

Survey Scan

IDA Criteria

Acquire MSMS Spectra

Dynamic Exclusion
IDA Workflows

- MultiTarget Screening (MTS)
  - MRM or sMRM survey scan will trigger EPI scans
  - High sensitivity

- General Unknown Screening (GUS)
  - EMS survey scan will trigger EPI scans
  - Searching for unknown compounds
EMS Triggering EPI for Library Search

EMS

Threshold

EPI

Library Search

intensity threshold
sMRM Quant Survey Triggering EPI for Library Search

Scheduled MRM™ Algorithm

Threshold

EPI

Library Search

intensity threshold
Cliquid® 3.0 Software
Cliquid® 4-Step Workflow

1. Choose a Test

2. Build Sample List

3. Select Report

4. Submit Samples
Automatic Report Generation
iMethod™ Test: Instant Methods for Accelerated Results

- Download a lab proven method from the web or a CD
- Add your samples
- Ready to go
http://www.absciex.com/imethods/
iMethod™ Tests

– Inborn Errors of Metabolism
– Antiretrovirals
– Vitamin D
– Immunosuppressants
– Steroids Panel
– Pain Panel
Summary

- LC-MS/MS provides the selectivity and sensitivity required to analyze patient samples for clinical research applications.

- LC-MS/MS allows for simultaneous quantitation and verification of results
  - Can detect non-volatile, polar and thermally labile compounds
  - More sensitive than GC, LC or GC/MS
  - More information for confirmation via RT, ion ratios, library searching

- LC-MS/MS has the ability to monitor hundreds of analytes per analysis
  - Monitor hundreds of analytes per analysis
  - Obtain full scan confirmatory MS/MS in the same run
  - High throughput capabilities – fast chromatography and multiple analytes can be screened for in the same run

- Cliquid Software and iMethod Tests provide ease of use along with a number of key features
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