*Clinical Chemistry* Trainee Council
Pearls of Laboratory Medicine
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**TITLE: Methodology: Mass Spectrometry**

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**Slide 1: Introduction**

This portion of the Pearls of Laboratory Medicine series will serve as an introduction to clinical mass spectrometry. Topics for discussion will include ionization methods, the different types of mass spectrometers used clinically, different methods of analysis, and highlight some future areas where mass spectrometry will most likely be applied.

**Slide 2: Typical Assay Overview**

In general, an assay that uses a mass spectrometer as the final detection method will involve a sample preparation step, a chromatographic separation and measurement with a mass spectrometer. For sample preparation this can be as simple as a dilution or as complex as solid phase extraction or immunoextraction. To obtain chromatographic separation, gas chromatography or liquid chromatography is most often used. The final stage of detection using a mass spectrometer is the focus of this portion of Pearls of Laboratory Medicine.

**Slide 3: Ionization**

In order for an analyte to be measured using mass spectrometry it must carry a charge and be in the gas phase. For analytes introduced into the mass spectrometer as a liquid, several types of sources exist including electrospray ionization and atmospheric pressure chemical ionization.

APCI uses a combination of heat to completely vaporize the sample and plasma produced by an electrical discharge. The electrical discharge first ionizes the evaporated solvent which in turn ionizes the analytes in the gas phase.

ESI sources use a combination of voltage, heat and air to produce successively smaller droplets from the liquid sample. As the drops become smaller, ion concentration increases until ions at the surface are ejected into the gas phase. For larger ions, such as proteins, the ions are liberated only after complete evaporation of the solvent.

If gas chromatography is used, the analyte is already in the gas phase and an electron ionization source is used to generate a charged analyte. Electron ionization uses a beam of electrons that interact with molecules to form radical cations, which in turn often undergo fragmentation in the source to more stable species.

### Slide 4: Mass-to-Charge Ratio

Once we have a way to get ions charged and into the gas phase, the most important aspect in the analysis is the mass-to-charge ratio, designated as m over z. An analyte with a molecular weight of 415 and a charge of +1 will have a mass-to-charge ratio of 415. Likewise, a molecule with a molecular weight of 15000 and a charge of +3 will have a mass to charge ratio of 500.

With the mass of our analyte known, we need to decide what type of mass spectrometer to use for analysis. Single quadrupole and triple quadrupole mass analyzers are the most common mass spectrometers found in the clinical laboratory, but it's important to recognize that laboratories are also using time of flight analyzers for specific applications.

Although every type of mass spectrometer uses the same charged ions, how it separates individual analytes varies greatly.

### Slide 5: Time-of-flight

Time-of-flight analyzers use an electric field to accelerate bundles of gas phase ions towards a detector. Since all ions are being accelerated by the same electric field, ions with a low mass-to-charge ratio will accelerate faster than ions with a high mass-to-charge ratio. How long it takes a given ion to travel down the flight tube is how ions of different mass-to-charge ratios are distinguished. TOF analyzers have essentially an unlimited mass-to-charge range, very high sensitivity, very high mass accuracy, high transmission percentage but a limited dynamic range.

### Slide 6: Linear Quadrupoles

Linear quadrupoles have four parallel rods arranged in a square formation. At any given time during the analysis, one set of diagonal rods is positive while the other diagonal set is negative. Which set is positive and which set is negative alternates back and forth during the analysis. For an analyte at a known mass-to-charge ratio, the amount of positive or negative charge and the frequency at which the diagonal pairs switch their sign is optimized to produce a successful ion flight path through the quadrupole. Each combination of voltage and frequency will result in some ions traveling through to the detector while others have unstable flight paths and ground out on a rod. How long the mass spectrometer sits at a voltage and frequency combination is called the dwell time, during which only a limited number of ions are successfully reaching the detector. Quadrupoles have a limited mass-to-charge range, high sensitivity, high mass accuracy, but a low ion transmission percentage.

### Slide 7: Tandem Mass Spectrometry

Tandem mass spectrometry is a method to further enhance the specificity of mass spectrometry. Ions pass through the first quadrupole, collide with inert gas in the collision cell producing fragments, and the fragments pass through the third quadrupole to the detector. Although several compounds may share the same molecular weight and therefore the same precursor ion, they can readily be distinguished using tandem mass spectrometry if unique product ions are produced in the collision cell.

 

**Slide 8: Method of Analysis: Full Scan, Selected Ion Monitoring and Multiple Reaction Monitoring**

For linear quadrupoles, full scanning is helpful in situations where the analytes of interest have yet to be determined. The resultant data is complex but the potential for discovering unknown components exists. The lack of specificity reduces the utility of this method for routine clinical testing. Importantly, with the use of a tandem mass spectrometer, scanning can be done in the first and third quadrupoles. Selected ion monitoring, referred to as SIM, can be used with known analytes of interest. For each analyte, an optimized voltage and frequency is determined. During analysis, the instrument continuously switches across each analyte-specific setting. This mode of analysis requires a single quadrupole and no fragmentation occurs.

Multiple reaction monitoring or MRM is used with tandem mass spectrometry. Similar to SIM, the analytes of interest are known and optimized voltage and frequency settings are determined for both precursor and resultant products. The specificity is enhanced with MRM because of the fragmentation that occurs prior to detection. Though analytes may often share the same molecular weight, it is less likely they will also share the same fragments. Using MRM, precursor ions with identical mass-to-charge ratios can easily be distinguished when unique product ions can be found.

**Slide 9: Challenges in Clinical Mass Spectrometry**

Ion suppression occurs when something in the sample interferes with the ionization process of the analytes. For example, nonvolatile or less volatile components found in the sample can inhibit proper droplet formation or efficiency of solvent evaporation. One method for determining ion suppression is a t-infusion experiment. Analyte is infused into the mass spectrometer at a constant rate while a sample is injected that does not contain the analyte but importantly is an appropriate matrix match. The sample tracing should be a constant line in the absence of ion suppression, but the relative abundance will appear to drop when ion suppression is occurring. Better sample preparation and improved chromatography are two possible solutions to reduce ion suppression.

Internal standards are used in many mass spectrometry assays as a means to address ion suppression and imprecision in the analysis. Correctly chosen internal standards will behave similarly to the analytes being measured but importantly must have a unique mass-to-charge ratio or for tandem mass spectrometry, a unique fragment. For example, an LC-MS method for tacrolimus and sirolimus might use ascomycin as an internal standard. The same amount of ascomycin is added to each sample and the resultant peak area of the analyte is divided by the peak area of the internal standard to provide a ratio used for further calculation.

**Slide 10: Future Applications in the Clinical Laboratory**

In the near future, the routine analysis of proteins will be an exciting addition to clinical mass spectrometry. Quantitative clinical proteomic methods using enzymatic digestion, isotope dilution for normalization and the use of MRM for analysis, distinguishes this from previous, shotgun proteomic methods.

Metabolic profiling promises yet another exciting addition to clinical mass spectrometry, linking genome and proteome alterations to the resultant metabolic disruption present in diseased states.

**Slide 11: References**