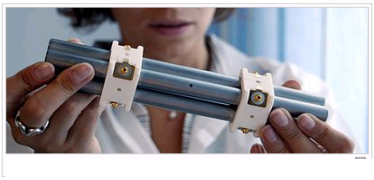
Development of an MRM Method

Infusion Pump (Harvard Apparatus)

•Delivers constant supply of sample

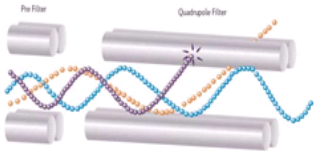
Mass Spectrometer (Sciex)

•Ionization Chamber
•Electromagnetic Lenses
•Triple Quadrupole
•Detector

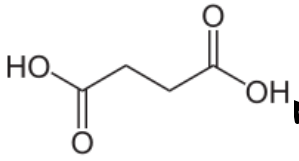


Quadrupole: An analyzer that stabilizes The trajectory of an ion based on the ion's Mass to charge ratio (*m/z*).

Mass to Charge Ratio: Shown as *m/z* where
m = ionized molecular weight of the molecule and z = charge.



Succinate molecules (blue) traveling between the quadrupoles at *m/z* = 117.



Succinic Acid

Negative mode ionization occurs when a proton is removed from a carboxylic acid group. The molecular weight of succinic acid is 118. The removal of a proton during negative mode ionization results in an *m/z* value of 117.

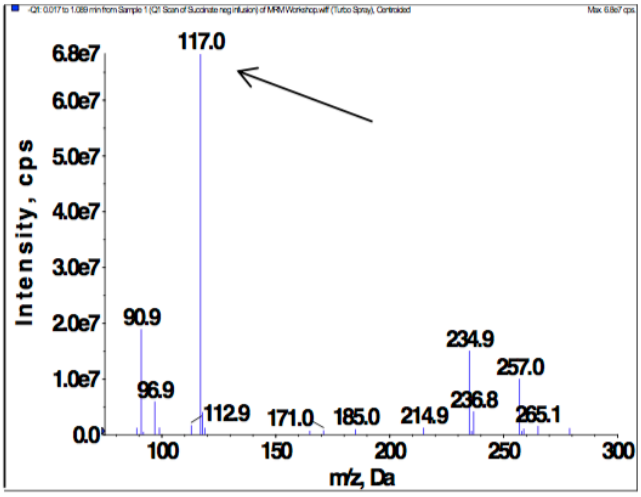
Most small molecules, such as succinate, are singly charged.

MRM Method Development Undergoes a Specific Process

1. Q1 Scan (survey scan) to verify the molecular weight of the compound

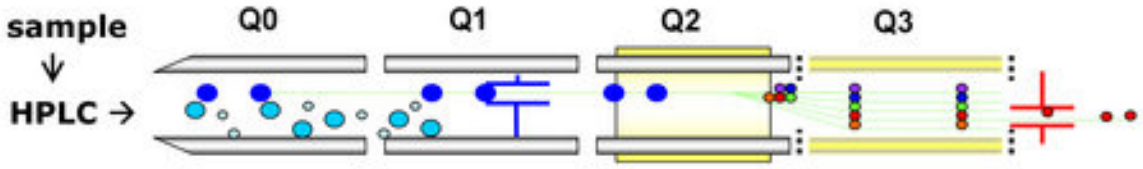
Succinate is displayed in the mass spectrum at *m/z* = 117

This type of scan will verify the **precursor ion**, also known as the **parent ion**.

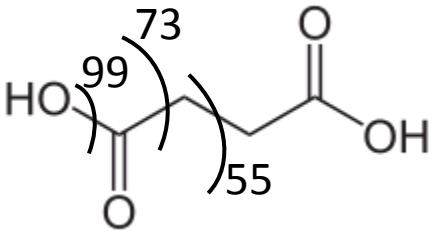


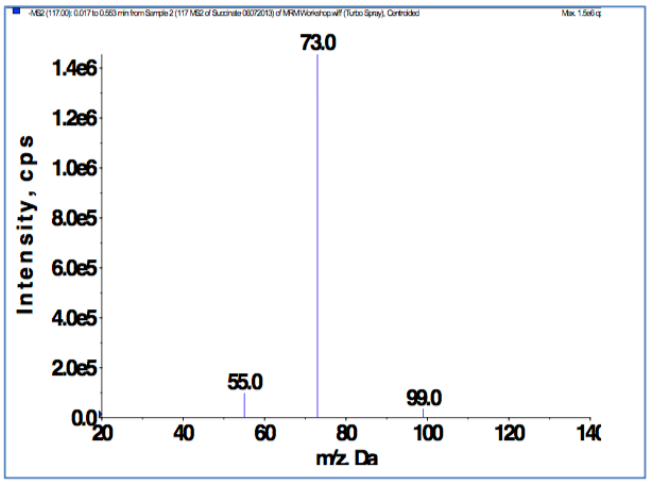
2. Fragmentation of Succinate

In a triple quadrupole mass spectrometer, ions are fragmented by accelerating them into a gas (in this case, nitrogen.) The precursor ion will break up into fragment ions of a lesser molecular weight. This process is called **fragmentation**.



The succinate ions (shown in blue) will be filtered away from other ions in Q1, accelerate into the collision gas and undergo fragmentation in Q2. All of the fragment ions will escape the collision cell (Q2) and enter Q3. In Q3, the fragment ions will be scanned for mass determination.



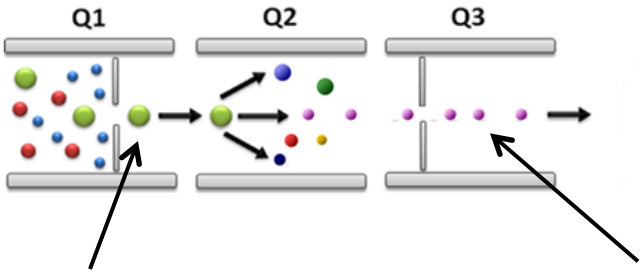


The most energetically favorable fragment is *m/z* = 73.
The mass transition for succinate will be 117/73.

For MRM Analysis, quadrupole 1 will lock onto *m/z* = 117 and quadrupole 3 will lock onto *m/z* = 73. Only fragment ions with an *m/z* of 73 will escape quadrupole 3 to strike the detector and generate a signal.

MRM: Multiple Reaction Monitoring

A mass spectrometry technique in which quadrupoles one and three are locked onto a specific mass transition.



Selected precursor (parent) ion for succinate *m/z* = 117

Secected fragment ion for succinate *m/z* = 73

Advantages of MRM Analysis:

Highly Specific
Low Background
Increased Sensitivity

Mass Transition Application to HPLC

The results of the survey scan and fragmentation ion scan are added together to produce what is called a mass transition. In a mass transition, quadrupole number one and three are locked onto specific *m/z* ratios. Quadrupole one stabilizes the trajectory of the intact charged molecule (parent ion) and quadrupole three stabilizes the trajectory of a selected fragment ion (daughter ion).

The mass transition enables the mass spectrometer to detect compounds (such as succinate) when they elute from a HPLC column into mass spectrometer.

Analysis using mass transitions is **called Multiple Reaction Monitoring** (MRM). MRM scans are extremely sensitive and Specific. They are commonly used to quantify molecules as they elute from an HPLC column.

In MRM method, MS spectrometer locks on specific mass transition to be able to detect metabolite of interest. Molecules elute from the column based on their hydrophobicity and enter the ionization chamber of the mass spectrometer.

Why can't you simply infuse samples for MRM analysis as we have done for succinic acid?

Advantages of combining mass spectrometry With HPLC

1. Automation
2. High Throughput
3. Sample integrity is preserved by the cooled autosampler