Development of an MRM Method

Infusion Pump (Harvard Apparatus)
•Delivers constant supply of sample
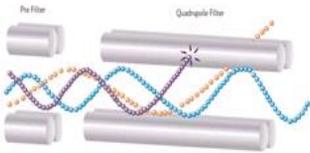
Mass Spectrometer (AB/Sciex)
•Ionization Chamber
•Electromagnetic Lenses
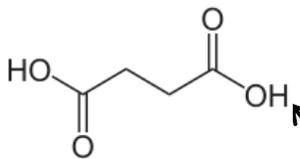•Triple Quadrupole
•Detector

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.

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
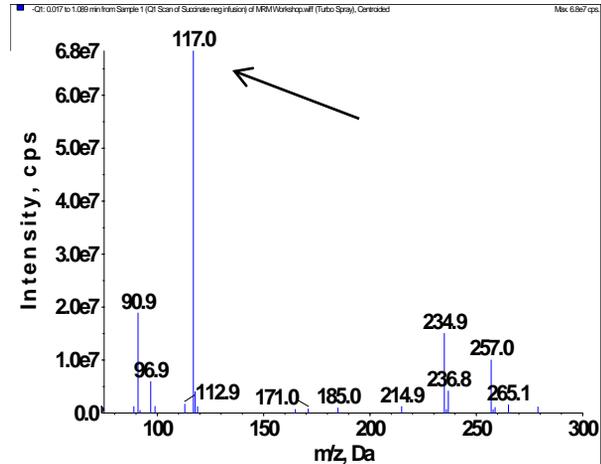ionization results in an m/z value of 117.

Succinic Acid

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

MRM Method Development Undergoes a Specific Process

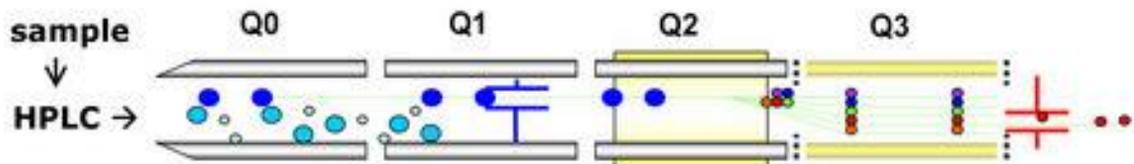1. Q1 Scan (survey scan) to verify the molecular weight of the compound

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

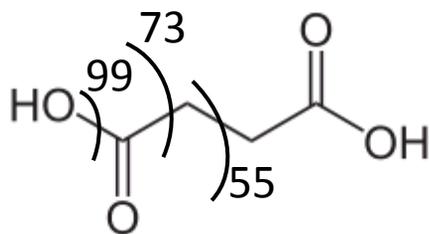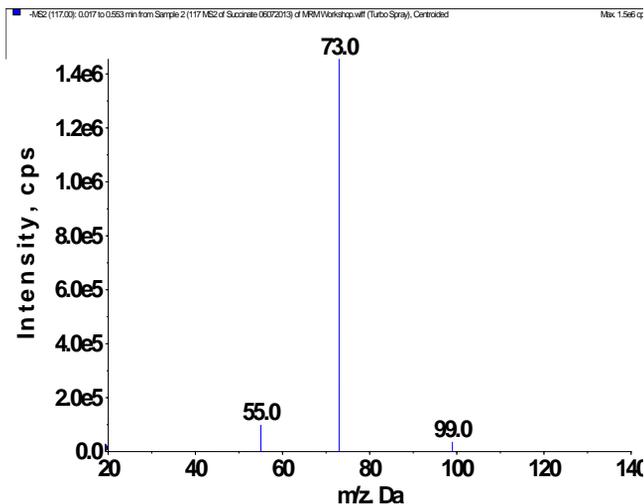This type of scan will verify the **precursor ion**, also known as the **parent ion**.

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.
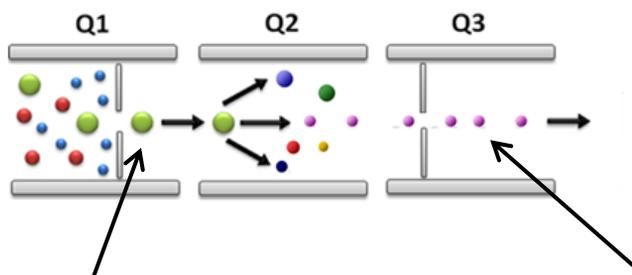
73.0

1.4e6

1.2e6

1.0e6

Intensity, cps

8.0e5

6.0e5

4.0e5

2.0e5

55.0

99.0

0.0

20   40   60   80   100   120   140

m/z, Da

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.

Q1   Q2   Q3

Selected precursor (parent) ion for succinate   **Secected fragment ion for succinate**
m/z = 117   **m/z = 73**

Advantages of MRM Analysis:
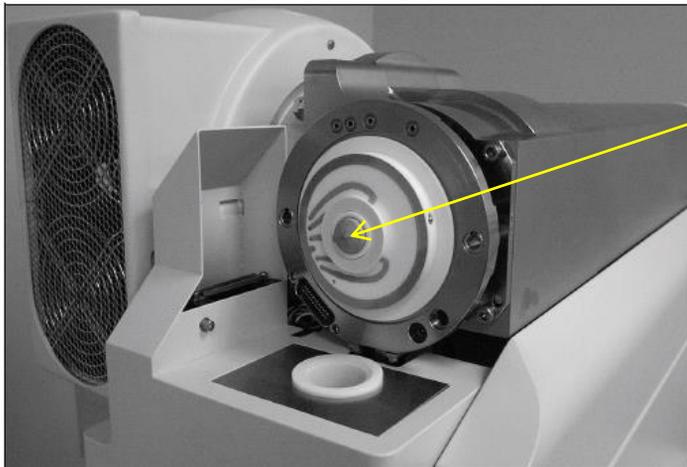**Highly Specific**
Low Background
Increased Sensitivity

As a part of this demonstration we will optimize the parameters required to detect succinate by LC-MS-MRM.

The following parameters on the mass spectrometer
will be optimized:

DP (declustering potential)
EP (entrance potential)
CE (collision energy)
CXP (collision cell exit potential)
CAD (collision gas)
IS (ionspray voltage)
TEM (temperature of ion source)
GS1 (nebulizing gas)
GS2 (drying gas)

_____ DP _____

Orifice (with ion source
and orifice cover removed)

The orifice is the opening where ions enter the mass spectrometer.
The declustering potential (**DP**) is a voltage applied to the orifice that helps to prevent the ions from clustering together.

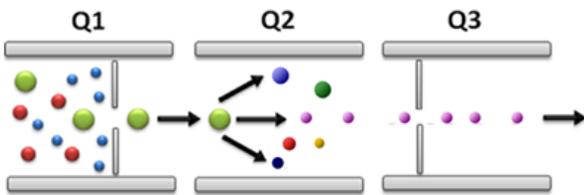The optimal DP for succinate is _____ volts.

_____ EP _____

View of the first set of quadrupoles (designated as **Q0** in the diagram on page 1) with the orifice plate removed. It is here that the entrance potential (**EP**) is applied.

The rods in Q0 do not act as mass filters but serve to guide and focus the ions into the mass spectrometer.

Ion path after entering orifice

The optimal entrance potential for succinate is _____ volts.
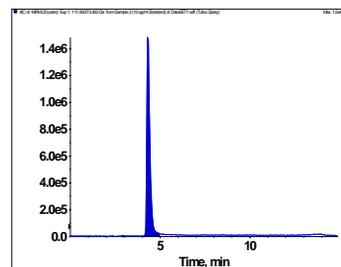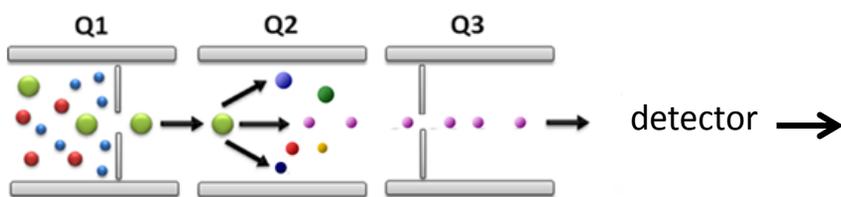

_____ CE _____

The higher the collision energy, the greater the fragmentation. If the CE is too low, the molecule undergoes inefficient fragmentation. Raising the CE too high can cause too much fragmentation. A CE set too high or low can drastically reduce the sensitivity of an MRM method.

The collision energy (**CE**) refers to the rate of acceleration as the ions enter quadrupole 2 (Q2.) Q2 is usually covered with a housing that allows it to maintain a small positive gas pressure while surrounded by high vacuum. The ions undergo a thermal interaction with the collision gas and fragment. In the example above, a molecule of succinate (green) enters Q2 at a sufficient velocity to undergo fragmentation.

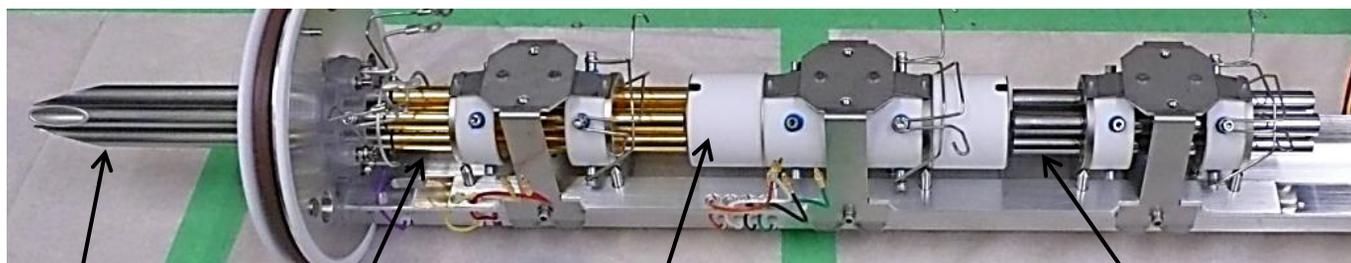The optimal collision energy for succinate is _____.

The Collision Cell Exit Potential (**CXP**) focuses and accelerates the ions out of Q2 and into Q3.

The CXP assists all of the fragmentation ions out of Q2 and into Q3. In the MRM method we are developing for succinate, all other fragment ions are filtered out of Q3 except for the fragment at m/z = 73. In the diagram above, only fragments at m/z = 73 (in purple) are allowed to strike the detector and generate a signal.

The optimal CXP for succinate is _____.

_____ CAD _____

Q0   **Q1 (gold plated)**   **Q2 (surrounded by housing)**   **Q3**

Collisionally Activated Dissociation (**CAD**) is the process of colliding precursor ions (parent ions) with a neutral gas to break the molecule into fragment ions. The neutral gas used during this demonstration is nitrogen.

The optimal CAD for succinate is _____.

The ionspray voltage (**IS**) is applied to the tip of the ionspray needle. It is here that the sample is ionized.

Gas 1 (**GS1**) is the nebulizer gas. It helps to generate small droplets that rapidly desolvate in the ion source.

GS1

Turbo V Source

IonSpray Voltage (IS)

Gas 2 (**GS2**) blows out of the ceramic heaters and helps to evaporate the spray droplets.

The additional temperature (**TEM**) added through the ceramic heaters helps to rapidly evaporate the spray droplets. Not all compounds require additional heat to ionize and in some cases the additional heat can be detrimental to sensitivity. The maximum temperature for the heaters is 750 ° C.

The optimal IS for succinate is _____ volts.

The optimal GS1 for succinate is _____ psi.

The optimal GS2 for succinate is _____ psi.

The optimal TEM for succinate is _____ ° C.

Please summarize the data you have recorded into the following table.

Precursor (parent) ion for succinate	
Selected fragment ion for succinate	
DP (declustering potential)	
EP (entrance potential)	
CE (collision energy)	
CXP (collision cell exit potential)	
CAD (collision gas)	
IS (ionspray voltage)	
TEM (temperature of ion source)	
GS1 (nebulizing gas)	
GS2 (drying gas)	

You have finished determining the parameters that we optimize for most MRM methods. In the next demo station (demo B) you will learn much more detail regarding MRM methods and their applications.