## 1. Define Composition of Blood, Plasma, Serum, Urine

#### **Blood:**

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells — such as nutrients and oxygen — and transports waste products away from those same cells. Blood accounts for 7% of the human body weight.

Elements of the blood are erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma constitutes about 54.3%, white cells constitute 0.7%.

#### Plasma:

Blood plasma is the yellow liquid component of blood, in which the blood cells in whole blood would normally be suspended. It makes up about 55% of the total blood volume. It is mostly water (92% by volume) and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide.

#### Serum:

Blood serum is blood plasma without fibrinogen or the other clotting factors (i.e., whole blood minus both the cells and the clotting factors).

#### Urine:

The fluid produced by the kidneys to remove waste products, excess water and other substances from the body.

A liquid containing multiple waste products of metabolism, especially urea and other nitrogenous compounds, that are filtered from the blood by the kidneys. Urine is stored in the urinary bladder and is excreted from the body through the urethra.

## 2. Write physicochemical properties helpful in starting the method development

pH, pKa, Ionization, nature of compound, Molarity, Normality.

# **3.** Solubility criteria for organic acid, base and neutrals, similarly for inorganic acids, base. (Which should get solublised in which medium)

Solvents and solutes can be broadly classified into polar (hydrophilic) and non-polar (lipophilic). The polarity can be measured as the dielectric constant or the dipole moment of a compound. The polarity of a solvent determines what type of compounds it is able to dissolve and with what other solvents or liquid compounds it is miscible with. As a rule of thumb, polar solvents dissolve polar compounds best and non-polar solvents dissolve non-polar compounds best: "like dissolves like". Strongly polar compounds like inorganic salts (e.g. table salt) or sugars (e.g. sucrose) dissolve only in very polar solvents like water, while strongly non-polar compounds like oils or waxes dissolve only in very non-polar organic solvents like hexane. Similarly, water and hexane (or vinegar and salad oil) are not miscible with each other and will quickly separate into two layers even after being shaken well.

## 4. Define liquid liquid extraction i.e. define partition coefficient

#### Liquid-liquid extraction:

Liquid-liquid extraction, also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase.

#### Partition coefficient and log P:

The Partition Coefficient itself is a constant. It is defined as the ratio of concentration of compound in aqueous phase to the concentration in an immiscible solvent, as the neutral molecule.

Partition Coefficient, P = [Organic] / [Aqueous] Where [] = concentration Log P= log10 (Partition Coefficient) <u>NOTE:</u> LogP = 1 means 10:1 Organic: Aqueous

LogP = 0 means 1:1 Organic: Aqueous Log P = -1 means 1:10 Organic: Aqueous The partition coefficient is a ratio of concentrations of un-ionized compound between the two solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is adjusted such that the predominant form of the compound is un-ionized. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log P:

$$log \ P_{oct/wat} = log igg( rac{[solute]_{octanol}}{[solute]_{water}^{un-ionized}} igg)$$

#### **Distribution coefficient and log D:**

Log D is the log distribution coefficient at a particular pH. This is not constant and will vary according to the protogenic nature of the molecule.

Distribution Coefficient,  $D = [Unionised]_{(a)} / [Unionised]_{(aq)} + [Ionised]_{(aq)}$ Log  $D = log_{10}$  (Distribution Coefficient) Log D is related to LogP and the pKa by the following equations:

$$\log D_{(pH)} = \log P - \log [1 + 10^{(pH-pK_{h})}]$$
 for acids

 $\text{Log}\,\mathbb{D}_{(pH)} \,=\, \log \mathbb{P} \, - \log [1 + 10^{(pKa-pH)}]_{for \ bases}$ 

The distribution coefficient is the ratio of the sum of the concentrations of all forms of the compound (ionized plus unionized) in each of the two phases. For measurements of distribution coefficient, the pH of the aqueous phase is buffered to a specific value such that the pH is not significantly perturbed by the introduction of the compound. The logarithm of the ratio of the sum of concentrations of the solute's various forms in one solvent, to the sum of the concentrations of its forms in the other solvent is called Log D:

$$log \ D_{oct/wat} = log \left( \frac{[solute]_{octanol}}{[solute]_{water}^{ionized} + [solute]_{water}^{neutral}} \right)$$

In addition, log D is pH dependent, hence the one must specify the pH at which the log D was measured. Of particular interest is the log D at pH = 7.4 (the physiological pH of blood serum). For un-ionizable compounds, log P = log D at any pH.

#### Ionization:

When pH = pKa then there is 50% ionization

$$HA = H^{+} + A^{-}$$

$$K_{a} = \frac{[H^{+}][A^{-}]}{[HA]}$$

$$-\log K_{a} = -\log [H^{+}] - \log \frac{[A^{-}]}{[HA]}$$

$$pK_{a} = pH - \log \frac{[A^{-}]}{[HA]}$$

At 50% ionization  $[A^-] = [HA]$  so  $\log \frac{[A^-]}{[HA]} = \log 1 = 0$ i.e. At 50% ionization  $pH = pK_a$ 

	PERCENTAG	E IONIZATION	ACIDS
pH-pKa	ACIDS	BASES	
4	0.1	99.9	%Ionization = $\frac{100}{\sqrt{100}}$
-2	1	99	$(pK_a - pH)$
-1	10	90	1410 \$
0	50	50	DAGEG
1	90	10	BASES
2	99	1	%Ionization = <u>100</u>
3	99.9	0.1	$\frac{1}{1+10} \left( pH - pK_a \right)$
			1+10(1-1-4)

Rules					
Acid drugs – become more NON ionized in acidic pH					
Basic drugs – become more NON ionized in basic pH (alkaline pH)					
Acid Drug Basic Drug					
	Acid Drug	Basic Drug			
Acid pH Environment	Acid Drug	Basic Drug			

#### pKa or dissociation constant:

The pKa or ionisation constant is defined as the negative logarithm of the equilibrium coefficient of the neutral and charged forms of a compound. This allows the proportion of neutral and charged species at any pH to be calculated, as well as the basic or acidic properties of the compound to be defined.

for acids:

 $HA = H^+ + A^-$ 

 $Ka = (H^{+})(A^{-})/(HA)$  Where  $\{ \}$  = activity in Mole litre-1

pKa = -log10(Ka)

for bases

 $BH^+ = H^+ + B$ 

 $Ka = (H^{+})(B)/(BH^{+})$ 

pKa = -log10(Ka)

## 5. Define the Principal of SPE

Solid Phase Extraction is performed by absorbing the analyte(s) from the matrix into a solid support (sorbent). SPE extract drug from biological fluids prior to quantitative analysis. SPE methods have four steps:

1	Step Conditioning step	Purpose To prepare the sorbent for effective interaction with the analyte(s) by solvation or activation of the ligands on the chromatographic surface, followed by equilibration in the solvent similar to the sample/matrix.
	a) Solvation step	Washed and wetted with methanol (organic solvent). To remove air trapped, and solvation or activation of the ligands on the chromatographic surface, enabling them to interact more effectively with target analyte(s).
	b) Equilibration step	Remove residual methanol (organic solvent) and equilibrates the sorbent in a solvent that will maximize the interactions with the target analyte(s)
2	Sample loading step	To adjust the sample/matrix composition (via dilution, etc.) such that the analyte(s) is quantitatively retained on the sorbent while the amount of bound impurities is minimized.
3	Washing step	To remove impurities that are bound to the sorbent less strongly than the analyte(s)
4	Elution step	To selectively desorb and recover the analyte(s) by disrupting the analyte- sorbent interactions.

#### **Type of SPE**

1. Reversed Phase:

"Reversed Phase" extractions are commonly used to extract hydrophobic or even polar organic analytes from an aqueous sample/matrix. Hydrocarbon chains on both the analyte and the sorbent are attracted to one another by low energy van der Waals dispersion force. Common reversed phase sorbents contain saturated hydrocarbon chains such as C18 and C8, or aromatic rings such as Phenyl (PH) or SDB. Because reversed phase extractions are relatively non-specific, a wide range of organic compounds is typically retained. As a result, it is important to optimize the extraction condition, particularly the composition of the wash solvent. Analytes are typically eluted with organic solvents such as methanol or acetonitrile, in combination with water, acids, bases, or other solvents and organic modifier.

2. Normal Phase:

"Normal phase" retention mechanisms are commonly employed to extract polar analytes from nonpolar organic solvents. The retention mechanism is based on hydrogen bonding, dipole-dipole and  $\pi$ - $\pi$  interaction between polar analytes and polar stationary phase such as silica, alumina and Florisil<sup>®</sup>. Highly specific normal phase extraction can be obtained by carefully optimizing the polarity of the conditioning solvent and the solvent(s) used to dilute and load the sample/matrix. Analytes can be eluted with the use of relatively low concentrations of polar organic solvents such as methanol or isopropanol, in combination with non-polar organic solvents.

3. Ion Exchange:

"ion exchange" mechanism are used to extract charged analytes from low ionic strength aqueous or organic samples. Charged sorbent are used to retain analytes of the opposite charge. For example, positively charged analytes containing amines are retained on negatively charged "cation exchangers" such as sulfonic or carboxylic acids. In contrast, negatively charged analytes containing sulfonic acid or carboxylic acid groups are retained on positively charged "anion exchangers" containing any one of a variety of different amino groups. Ion exchange mechanisms rely on specific, high-energy coulombic interactions between the sorbent and the analyte. Only species of the proper charge are retained by the column, so most matrix contaminants are simply rinsed away to waste during the loading and the wash steps. For this reason, cation exchange SPE is commonly used for the extraction of basic compounds (drugs and other amines) from complex biological samples. Analytes are typically eluted with high ionic strength salts and buffers and/or strong acids or bases.

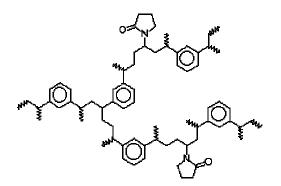
#### Oasis® HLB (Hydrophilic-Lipophilic-Balanced)

An exceptionally clean, highly reproducible, patented copolymer synthesized with a unique composition that is hydrophilic-lipophilic-balanced for both strong reversed-phase retention and water-wettability. Compatible with sample or eluents from pH 1 to 14.

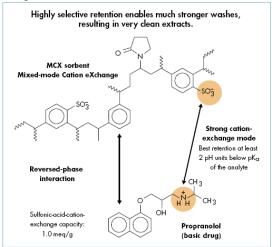
Used to adsorb both polar and non-polar compounds simultaneously from aqueous media; typical applications include drugs and their metabolites from biological fluids, environmental pollutants from water.

HLB can be substituted for, has a wider spectrum of retention, and is more reproducible than  $C_{18}$  and all other silicaor polymer-based reversed-phase media. Oasis<sup>®</sup> HLB is the ideal starting point for new reversed-phase SPE method development.

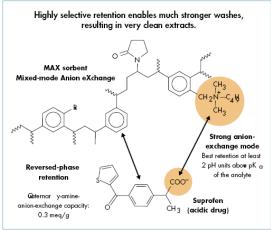
**Pore Size (nominal):** 80 Å **Particle Size:** 30 μm [or 60 μm for LP grade] **Surface Functionality:** m-Divinylbenzene & N-vinylpyrrolidone copolymer



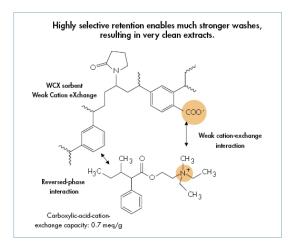
- Oasis HLB: Hydrophilic-Lipophilic Balance Sorbent reversed-phase sorbent for all compounds (e.g. parent drug and its polar metabolites). Water-wettable sorbent, no impact of sorbent drying. One sorbent, one method for all of your general SPE needs.
- Oasis MCX: Mixed-mode Cation-eXchange and reversed-phase sorbent for bases. High selectivity for basic compounds.



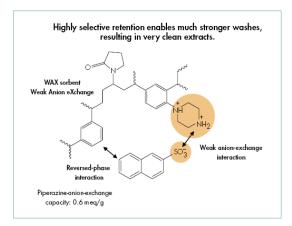
• Oasis MAX: Mixed-mode Anion-eXchange and reversed-phase sorbent for acids. High selectivity for acidic compounds.



• Oasis WCX: mixed-mode Weak Cation-eXchange and reversed-phase sorbent. Retain and release strong bases (e.g. quaternary amines).



• Oasis WAX: mixed-mode Weak Anion-eXchange and reversed-phase sorbent. Retain and release strong acids (e.g. sulfonates).



## Ion-Exchange Guidelines

First, determine analyte type.

Then, follow corresponding arrows down for recommended particle and mobile phase pH.

Analyte Type		ACID Ka ~ 5	Strong ACID		k BASE Ka ~ 10	Strong BASE
Charge State vs. pH*	No charge at pH < 3	anion] at pH > 7	[anion] Always Charged	+ [cation] at pH < 8	No Charge of pH > 12	+ [cation] Always Charged

Stationary Phase Particle	Strong Anion Exchanger	Weak Anion Exchanger e.g., pKa - 10	Strong Cation Exchanger	Weak Cation Exchanger e.g., pKg - 5
Charge State vs. pH*	+ Always Charged	+ No Charge pH < 8 pH > 12	 Always Charged	No — Charge at at pH > 7 pH < 3
Mobile Phase pH Range				
to Retain analyte [capture]	pH > 7	pH < 8	pH < 8	pH > 7
to Release analyte [elute]	pH < 3	pH > 12	pH > 12	pH < 3

\*Note: pH Ranges are approximate. They will depend upon specific analyte and particle characteristics.

## SPE Method Development Summary

The following table summarizes the foregoing discussion of the modes of SPE:

Summary of Utility and Practice of Principal LC Modes for Solid-Phase Extraction [SPE]

	<b>Reversed Phase</b>	Normal Phase	Ion Exchange	
Analyte	Moderate to low polarity	Low to high polarity/neutral	Charged or Ionizable	
Separation Mechanism	Separation based on hydrophobicity	Separation based on polarity	Separation based on	charge
Sample Matrix	Aqueous	Non-polar organic solvent	Aqueous/ Low ionic	strength
Condition/ Equilibrate SPE Sorbent	<ol> <li>Solvate with polar organic</li> <li>Water</li> </ol>	Non-polar organic	Low ionic strength b	uffer
Preliminary Wash Step	Aqueous/buffer	Non-polar organic	Low ionic strength b	ouffer
Elution Steps	Increase polar organic content	Increase eluotropic strength of organic solvent mixture	Stronger buffers - ionic strength or pH to neutralize the charge	
			AX [Anion Exchange]	CX [Cation Exchange]
Sorbent Functionality	C <sub>18</sub> , tC <sub>18</sub> , C <sub>8</sub> , tC <sub>2</sub> , CN, NH <sub>2</sub> , HLB, RDX, Rxn RP	Silica, Alumina, Florisil, Diol, CN, NH <sub>2</sub>	Accell Plus QMA, NH <sub>2</sub> , SAX, MAX, WAX	Accell Plus CM, SCX, MCX, WCX, Rxn CX
Sorbent Surface Polarity	Low to Medium	High to Medium	High	High
Typical Solvent Polarity Range	High to Medium	Low to Medium	High	High
Typical Sample Loading Solvent	Water, low strength buffer	Hexane, chloroform, methylene chloride	Water, low strength buffer	Water, low strength buffer
Typical Elution Solvent	CH <sub>3</sub> OH/water, CH <sub>3</sub> CN/water	Ethyl acetate, acetone, CH <sub>3</sub> CN	Buffers, salts with high ionic strength, increase pH	Buffers, salts with high ionic strength, decrease pH
Sample Elution Order	Most polar sample components first	Least polar sample components first	Most weakly ionized sample component first	Most weakly ionized sample component first
Mobile Phase Solvent Change Required to Elute Compounds	Decrease solvent polarity	Increase solvent polarity	Increase ionic strength or increase pH	Increase ionic strength, or lower pH

This has been a brief introduction to sample enrichment and purification using solid-phase extraction [SPE]. The best way to start using SPE is to first learn what others have done with analytes and/or matrices similar to those of interest to you.

## 6. Define HPLC

High-performance liquid chromatography (HPLC) is a form of liquid chromatography used to separate, identify, and quantify compounds. HPLC utilizes a column that holds chromatographic packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.

#### **Reverse-phase**

Partition chromatography uses a relatively nonpolar stationary phase and a polar mobile phase, such as methanol, acetonitrile, water, or mixtures of these solvents. The most common bonded phases are n-octyldecyl (C18) and n-decyl (C8) chains, and phenyl groups. Reverse-phase chromatography is the most common form of liquid chromatography, primarily due to the wide range on analytes that can dissolve in the mobile phase.

An elution procedure used in liquid chromatography in which the mobile phase is significantly more polar then the stationary phase, e.g., a microporous silica-based material with chemically bonded alkyl chains.

#### Normal-phase

partition chromatography uses a polar stationary phase and a nonpolar organic solvent, such as n-hexane, methylene chloride, or chloroform, as the mobile phase. The stationary phase is a bonded siloxane with a polar functional group. The most common functional groups in order of increasing polarity are:

cyano:	-C2H4CN
diol:	-C3H6OCH2CHOHCH2OH
amino:	-C3H6NH2

dimethylamino: -C3H6N(CH3)2

An elution procedure in which the stationary phase is more polar than the mobile phase. This term is used in liquid chromatography to emphasize the contrast to reversed-phase chromatography.

	Separation mechanisms in LC				
adsorption	selective adsorption/desorption on a solid phase				
partition	selective partition between two immiscible liquids				
ion-exchange	differences in ion-exchange properties				
ion-pair	formation of ion-pair and selective partition or sorption of these ion-pairs				
gel permeation / size exclusion	differences in molecular size, or more explicitly the ability to diffuse into and out of the pore system				

	Phase systems in various LC modes				
Mechanism Mobile phase Stationary p					
adsorption (normal-phase)	apolar organic solvent with organic modifier	silica gel, alumina bonded-phase material			
adsorption (reversed-phase)	aqueous buffer with organic modifier, <i>e.g.</i> , CH <sub>3</sub> OH or CH <sub>3</sub> CN	bonded-phase material, <i>e.g.</i> , octadecyl-modified silica gel			
ion-pair	aqueous buffer with organic modifier and ion-pairing agent	reversed-phase bonded- phase material			
partition	liquid, mostly nonpolar	liquid, physically coated on porous solid support			
ion exchange	aqueous buffers	cationic or anionic exchange resin or bonded-phase material			
size exclusion	non-polar solvent	silica gel or polymeric material			

## 7. Define Bioavailability, bioequivalent and Clinical Trials

#### **Bioavailability**

Bioavailability means the rate and extent to which the active substance or therapeutic moiety is absorbed from a pharmaceutical form and becomes available at the site of action. In the majority of cases substances are intended to exhibit a systemic therapeutic effect, and a more practical definition can then be given, taking into consideration that the substance in the general circulation is in exchange with the substance at the site of action:

- Bioavailability is understood to be the extent and the rate to which a substance or its therapeutic moiety is delivered from a pharmaceutical form into the general circulation.

It may be useful to distinguish between the "absolute bioavailability" of a given pharmaceutical form as compared with that (100%) following intravenous administration, and the "relative bioavailability" as compared with another form administered by any route other than intravenous (e.g. tablets v. oral solution).

#### Bioequivalent

Two medicinal products are bioequivalents if they are pharmaceutical equivalents or alternatives and if their bioavailabilities (rate and extent) after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same.

"Bioequivalence" is a comparison of the bioavailability of two or more drug products.

#### **Clinical Trials**

Clinical trials are conducted in phases.

Phase 1 trials try to determine dosing, document how a drug is metabolized and excreted, and identify acute side effects. Usually, a small number of healthy volunteers (between 20 and 80) are used in Phase 1 trials.

Phase 2 trials include more participants (about 100-300) who have the disease or condition that the product potentially could treat. In Phase 2 trials, researchers seek to gather further safety data and preliminary evidence of the drug's beneficial effects (efficacy), and they develop and refine research methods for future trials with this drug. If the Phase 2 trials indicate that the drug may be effective--and the risks are considered acceptable, given the observed efficacy and the severity of the disease--the drug moves to Phase 3.

In Phase 3 trials, the drug is studied in a larger number of people with the disease (approximately 1,000-3,000). This phase further tests the product's effectiveness, monitors side effects, and, in some cases, compares the product's effects to a standard treatment, if one is already available. As more and more participants are tested over longer periods of time, the less common side effects are more likely to be revealed.

Sometimes, Phase 4 trials are conducted after a product is already approved and on the market to find out more about the treatment's long-term risks, benefits, and optimal use, or to test the product in different populations of people, such as children.

#### **Time Release Technology**

Time Release Technology also known as Sustained-release (SR), extended-release (ER, XR, or XL), time-release or timed-release, controlled-release (CR), or continuous-release (CR or Contin) pills are tablets or capsules formulated to dissolve slowly and release a drug over time. The advantages of sustained-release tablets or capsules are that they can often be taken less frequently than instant-release formulations of the same drug, and that they keep steadier levels of the drug in the bloodstream. The first Sustained release tablets were made by Howard Press, in Hoboken, NJ in the early 50's and the first tablets relased under his process patent were called "Nitroglyn" and made under license by Key Corp., in Florida. Today most are formulated so that the active ingredient is embedded in a matrix of insoluble substance (various: some acrylics, even chitin, these are often patented) so that the dissolving drug has to find its way out through the holes in the matrix. In some SR formulations the matrix physically swells up to form a gel, so that the drug has first to dissolve in matrix, then exit through the outer surface.

There are certain considerations for the formation of sustained release formulation:

• If the active compound has a long half-life (over 6 hours), it is sustained on its own.

- If the pharmacological activity of the active compound is not related to its blood levels, time releasing then has no purpose.
- If the absorption of the active compound involves an active transport, the development of a time-release product may be problematic.
- Finally, if the active compound has a short half-life, it would require a large amount to maintain a prolonged effective dose. In this case, a broad therapeutic window is necessary to avoid toxicity; otherwise, the risk is unwarranted and another mode of administration would be recommended.

The difference between controlled release and sustained release is that controlled release is a perfectly zero order release; that is, the drug releases over time irrespective of concentration. Sustained release implies slow release of the drug over a time period. It may or may not be controlled release.

## 8. Difference between selectivity and specificity

Selectivity refers to a method that gives responses for a number of substances and can distinguish the analyte(s) response from all other response. Specificity refers to a method that gives response for only one single analyte

## 9. Good laboratory practice principles (GLP)

Good Laboratory Practice (GLP) is a quality system concerned with the organisational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

Fundamental rules incorporated in OECD guidelines and national regulations concerned with the process of effective organization and the conditions under which laboratory studies are properly planned, performed, monitored, recorded, and reported.

## **10.What is Good Clinical Practice (GCP)?**

"Good clinical practice is a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects."

## 11. What types of Common Ingredient use to prepare Mobile Phase / Buffers in MS?

The following lists the most common ingredients used to prepare mobile phases/buffers for reverse-phase LC/MS (API):

- Water
- Methanol
- Acetonitrile
- Formic acid (<0.1%)
- Acetic acid (<0.1%)
- Trifluoroacetic acid (<0.1%)
- Ammonium acetate (<10 mM)
- Ammonium formate (<10 mM)
- Ammonium hydroxide
- Triethylamine (TEA)

### 12.Define Principal of UV-Visible detector and its range of working.

UV-visible detectors operate on the principle that the analytes of interest absorb light in the UV or visible region of the electromagnetic spectrum

UV detectors function on the capacity of many compounds to absorb light in the wavelength range 180 to 350 nm.

## 13.Difference between high pressure and low pressure pump function.

#### The High Pressure Gradient

There are two basic types of solvent programmer. In the first, the solvent mixing occurs at high pressure and in the second the solvents are premixed at low pressure and then passed to the pump. The high pressure programmer is the simplest but most expensive as each solvent requires its own pump. Theoretically, there can be any number of solvents involved in a mobile phase program, however, most LC analyses require only two solvents, nevertheless, up to four solvents can be accommodated. The layout of a high pressure gradient system is shown in figure 2 and includes, as an example, provision for three solvents to be mixed by appropriate programming. Solvent passes from each reservoir directly to a pump and then to a mixing manifold from which it passes to the sample valve and column. The pumps control the actual program and are usually driven by stepping motors.

#### The Low Pressure Gradient

In a low pressure programmer, the solvent from each reservoir passes to an oscillating valve, the output from which is connected to a mixing manifold. The manifold receives and mixes solvents from each of the programmed valves. The valves are electrically operated and programmed to open and close for different periods of time by adjusting the frequency and wave form of the supply. Thus, a pre-determined amount of each solvent is allowed to flow into the manifold. The valves can also be driven either by oscillators contained in a separate electronic programmer or by the chromatograph computer which modifies the wave form and frequency to control the flow of each solvent.

#### 14. Mass Spectrometry - a definition

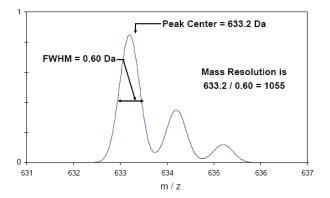
Mass spectrometry is the study of systems causing the formation of gaseous ions, with or without fragmentation, which are then characterised by their mass to charge ratios (m/z) and relative abundances.

Mass spectrometry is a technique for separating and identifying molecules based on mass.

The MS principle consists of ionizing chemical compounds to generate charged molecules or molecule fragments and measurement of their mass-to-charge ratios.<sup>[</sup>

## **15.What is mass resolution?**

Mass resolution is usually expressed as:  $m/\Delta m$ 



Where m is the m/z centroid of the peak and  $\Delta m$  is the width of the peak at 5% or more commonly, 50% of the maximum FWHM (Full-Width Half-Maximum)

### 16.What is ADME?

ADME is an acronym in pharmacokinetics and pharmacology for absorption, distribution, metabolism, and excretion, and describes the disposition of a pharmaceutical compound within an organism. The four criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug.

#### Absorption/Administration

For a compound to reach a tissue, it usually must be taken into the bloodstream - often via mucous surfaces like the digestive tract (intestinal absorption) - after being taken up by the target cells. This can be a serious problem at some natural barriers like the blood-brain barrier. Factors such as poor compound solubility, gastric emptying time, intestinal transit time, chemical instability in the stomach, and inability to permeate the intestinal wall can all reduce the extent to which a drug is absorbed after oral administration. Absorption critically determines the compound's bioavailability. Drugs that absorb poorly when taken orally must be administered in some less desirable way, like intravenously or by inhalation (e.g. zanamivir).

#### Distribution

The compound needs to be carried to its effector site, most often via the bloodstream. From there, the compound may distribute into tissues and organs, usually to differing extents. After entry into the systemic circulation, either by intrascular injection or by absorption from any of the various extracellular sites the drug is subjected to a number of process called as distribution process that tend to lower its plasma concentration. Distribution is defined as the reversible transfer of a drug between one compartment to another. Some factors affecting distribution include blood flow rates and the drug binding to serum proteins forming a complex.

#### Metabolism

Compounds begin to break down as soon as they enter the body. The majority of small-molecule drug metabolism is carried out in the liver by redox enzymes, termed cytochrome P450 enzymes. As metabolism occurs, the initial (parent) compound is converted to new compounds called metabolites. When metabolites are pharmacologically inert, metabolism deactivates the administered dose of parent drug and this usually reduces the effects on the body. Metabolites may also be pharmacologically active, sometimes more so than the parent drug.

#### **Excretion/Elimination**

Compounds and their metabolites need to be removed from the body via excretion, usually through the kidneys (urine) or in the feces. Unless excretion is complete, accumulation of foreign substances can adversely affect normal metabolism.

There are three sites where drug excretion occurs. The kidney is the most important site and it is where products are excreted through urine. Biliary excretion or faecal excretion is the process that initiates in the liver and passes through to the gut until the products are finally excreted along with waste products or faeces. The last method of excretion is through the lungs e.g. anaesthetic gases.

Excretion of drugs by the kidney involves 3 main mechanisms:

- Glomerular filtration of unbound drug.
- Active secretion of (free & protein-bound) drug by transporters e.g. anions such as urate, penicillin, glucuronide, sulfate conjugates) or cations such as choline, histamine.
- Filtrate 100-fold concentrated in tubules for a favourable concentration gradient so that it may be reabsorbed by passive diffusion and passed out through the urine.

### **17.What is Metabolism?**

Metabolism is the set of chemical reactions that occur in living organisms to maintain life. These processes allow organisms to grow and reproduce, maintain their structures, and respond to their environments. Metabolism is usually divided into two categories. Catabolism breaks down organic matter, for example to harvest energy in cellular respiration. Anabolism, on the other hand, uses energy to construct components of cells such as proteins and nucleic acids.

#### Catabolism

Reactions involving the breaking down of organic substrates, typically by oxidative breakdown, to provide chemically available energy (e.g. ATP) and/or to generate metabolic intermediates used in subsequent anabolic reactions. Catabolism is the set of metabolic pathways that break down molecules into smaller units and release energy.[1] In

catabolism, large molecules such as polysaccharides, lipids, nucleic acids and proteins are broken down into smaller units such as monosaccharides, fatty acids, nucleotides and amino acids, respectively. As molecules such as polysaccharides, proteins and nucleic acids are made from long chains of these small monomer units (mono = one + mer = part), the large molecules are called polymers (poly = many).

#### Anabolism

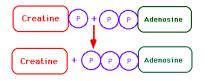
The processes of metabolism that result in the synthesis of cellular components from precursors of low molecular weight. Anabolism is the set of metabolic pathways that construct molecules from smaller units.[1] These reactions require energy. One way of categorizing metabolic processes, whether at the cellular, organ or organism level is as 'anabolic' or as 'catabolic', which is the opposite. Anabolism is powered by catabolism, where large molecules are broken down into smaller parts and then used up in respiration. Anabolic processes tend toward "building up" organs and tissues. These processes produce growth and differentiation of cells and increase in body size, a process that involves synthesis of complex molecules. Examples of anabolic processes include the growth and mineralization of bone and increases in muscle mass.

#### **Five Types of Metabolic Reactions**

The metabolic reactions in your body fall into five broad types and understanding these will make it much easier for you to understand the important metabolic reactions that happen in organisms.

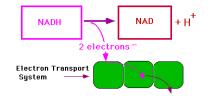
#### 1) Functional group transfer

A common type of reaction is a functional group transfer from one compound to another. For example in your muscles, during muscle activity creatine phosphatewill give up its phoshate to ADP (Adenosine di-phosphate) resulting in Creatine and ATP (Adenosine tri-phophate).



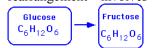
#### 2) Electron transfer

Electrons carry energy and this energy is released by transfering electrons from one substance to another, for example from an electron carrier to some type of protein system that can transport electrons and use the electron's energy to do work. For instance, the illustration shows NADH an electron carrier being broken down into NAD+ and H+ along with 2 electrons. These electrons are picked up by a system of proteins in the plasma membrane of certain organelles. These proteins then pass the electrons along from one to the other resulting in energy being released to do work. Often this work involves making ATP but in the case of bacteria the work may be to move the organism around its environment.



#### 3) Rearrangement

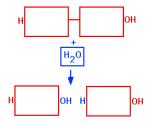
Many times a molecule's chemical structure will be rearranged into another molecule that has the same empirical formula(i.e., same number of atoms of each element). The result of such a rearrangement is a molecule with approriate physical and chemical properties for some other set of reactions. For instance, during cellular respiration glucose is combined with phosphates, transferred from ATP and rearranged to form fructose 1-6 bi-phosphate. Note that the rearrangement involves an input of energy which later on allows more energy to be harvested.



#### 4) Cleavage

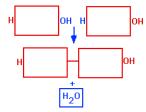
Cells and organisms in general, take large molecules and break them down into smaller molecules. For instance, when you eat a steak and then digest the proteins, the proteins are broken down into amino acids. The most common form of

cleavage is called hydrolysis because it involves essentially adding water to the bounds joining the small subunits of larger molecules, breaking those bonds.



#### 5) Condensation

The small molecules resulting from cleavage can then be used by the cell to make just the complex molecules it needs. For example, the amino acids from a steak or other protein source are recombined in protein synthesis into just the prooteins the cell needs and these may be quite different in function from the original muscle proteins in the steak. The common type of condensation reactions in cells is called dehydration synthesis because it involved removing a hydroxyl group from one molecule and a hydrogen from the other molecule when the two molecules are joined resulting in water as a by-product.

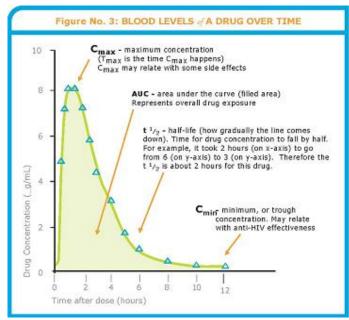


V	ersus ESI (Water	s) APCI	Electrospray
	Ionization	Gas phase process	Solution phase process
	Probe	Fused silica capillary	Stainless steel capillary
	Potential	Applied to corona pin	Applied to capillary
	Process	Probe heater vaporizes the liquid	Spray of charged droplets produced
		All molecules are now in the gas phase	Liquid is evaporated from the droplets
		Corona pin produces nitrogen ions	Then droplets split into smaller droplets
		Molecules are ionized when they collide with the nitrogen ions	When the droplets get small enough, ions enter the gas phase
	Fragments	More vigorous ionization, more fragments produced	Gentler ionization, less fragments produced
	Sample Types	Low MW<1000 Can be less polar	Small and large molecules tend to be more polar
	Charges	Usually singly charged	May be multiply charged
	Flow Rates	0.2 to 2 mL/min	0.001 to 1 mL/min
	Temperatures	Source ~ 120-140 °C Probe ~ 450-550 °C	Infusion:Source ~ 80 °C Desolvation ~ 120°CHPLC:Source ~ 120 °C Desolvation ~ 350 °C

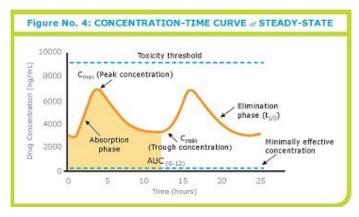
## **18.Different between APCI versus ESI?** APCI versus ESI (Waters)

### **19.PK Definitions**

There are certain terms and tests that researchers or doctors use when they study PK. The following is a summary of these PK measurements and what they mean. Please refer to Figures 3 and 4 for a picture of what all these PK measurements represent.



Above are blood levels (Y-axis) of a drug over time (X-axis) after a patient takes a single dose. In this representation, the patient took the dose at time 0 and would be due for another dose at time 12 (hours). Since the time 0 level is about equal to the time 12 level, the patient is at steady state. For AUC measurements, blood levels are high  $C_{max}$  is thought to increase the risk of side effects usually collected every hour or so. Figure No. 4 below is another way of looking at these same concepts.



AUC (area-under-the-curve): This is the overall amount of drug in the bloodstream after a dose. AUC studies are often used when researchers are looking for drug-drug or drug-food interactions. The way to get an AUC involves collecting many blood samples (usually every one or two hours) right after a person takes a dose up until the next dose is due. In each blood sample, the concentration of the drug is measured with a machine (discussed later). Then all the drug concentrations are put onto a graph based on the time after the dose that they were collected. A curve is made by connecting the points on the graph. The AUC for that drug is then calculated as the area under this drug concentration curve. An AUC study contains a lot of information about PK. It is probably the best way to understand how people handle a drug (PK).

**Cmax (maximum concentration):** This is the highest concentration of drug in the blood that is measured after a dose. C<sub>max</sub> usually happens within a few hours after the dose is taken. The time that C<sub>max</sub> happens is referred to as T<sub>max</sub>. For some antiretroviral drugs, a from the drug.

Cmin or trough (pronounced "troff") (minimum concentration): This is the lowest concentration of the drug in the blood that is measured after a dose. It happens right before a patient takes the next usual dose. It is not known for certain, but many people in the HIV community believe that keeping the trough concentration (Cmin) above a certain level is especially important for anti-HIV activity.

**Half-life** ( $t^{1/2}$ ): This is the amount of time it takes for the drug concentration in the blood to decline by half. The half-life is among the most important PK measurements for how often a drug has to be dosed (once-a-day or twice-a-day, etc).

**Steady-state:** This means that a person has been on a drug for enough time (usually one to two weeks) so that the drug concentration is not building up in the bloodstream anymore. The time it takes to get to steady-state depends on the half-life of the drug. A drug gets to steady state in about five half-lives.

As an illustration, before a patient reaches steady-state, each additional dose may be building the drug up in the body so each dose would be giving a higher Cmax, Cmin, and AUC. But, at steady-state, every dose would give the same Cmax, C<sub>min</sub>, and AUC in the patient because it is not building up any more.

Adherence: Remarkably, antiretroviral regimens lose effectiveness even with a small drop from perfect (or nearperfect) adherence. For example, going from 95?100% adherence down to 90?95% adherence with protease inhibitors resulted in a drop in effectiveness (viral load below 400) from 81% to 64%. It seems that the usual drug levels are not much higher than what?s needed for sustained efficacy. Additionally, the half-lives of the agents must have been relatively fast, such that the drug exposure fell below a level associated with a high probability of efficacy after the missed dose. Obviously, taking as close to 100% of antiretroviral doses is critically important.

**Once-a-day dosing:** Once daily combination antiretroviral therapies is a newer concept that is targeted to improve adherence. Several once-daily regimens are now available where all drugs have similar dietary requirements so that the whole regimen can be taken at the same time (see Figure 7: Options for Once-daily Dosing). It should be noted that only approved once-daily combinations should be used at this time (such as Truvada plus Sustiva as initial therapy). Some other antiretrovirals are currently approved for twice-a-day dosing, but they are being studied as once-a-day drugs. These "investigational" regimens should only be used in very controlled settings (like in a study). This is because it is not yet known if "investigational" drugs provide the right amount of drug exposure for effective and safe once-daily dosing (especially if a dose is missed). Which is better -- once-a-day dosing, they come out equal at the end.

 Sustiva

 Viread + Emtriva

 Viread + Epivir

 Ziagen + Epivir

 Viread + Videx

 Videx + Emtriva

 Videx + Epivir

 Videx + Epivir

 Videx + Epivir

**Pharmacodynamics (PD):** PD is just a fancy term for drug efficacy and toxicity. PD refers to what the drugs do to the human body. For example, HIV drugs cause HIV viral load to decline and CD4 cells to increase. Also, drugs sometimes cause certain side effects and toxicity in the human body.

## 20. Why vacuum needed in MS?

- Minimize ion-molecule collisions (that is, to maximize the mean free path).
  - Collisions can cause ions to deviate from the desired source-to-detector path.
  - Collisions can cause unplanned ion fragmentation or reaction.
- Prevent electrical arcing at kilovolt potentials needed for some ion focusing.
- Reduce contamination and chemical noise.

## **21.Fragmentation**

The molecular ions are energetically unstable, and some of them will break up into smaller pieces. The simplest case is that a molecular ion breaks into two parts - one of which is another positive ion, and the other is an uncharged free radical.

M; 🕨 X+ + Y•

A free radical is an atom or group of atoms which contains a single unpaired electron.

The uncharged free radical won't produce a line on the mass spectrum. Only charged particles will be accelerated, deflected and detected by the mass spectrometer. These uncharged particles will simply get lost in the machine - eventually, they get removed by the vacuum pump.

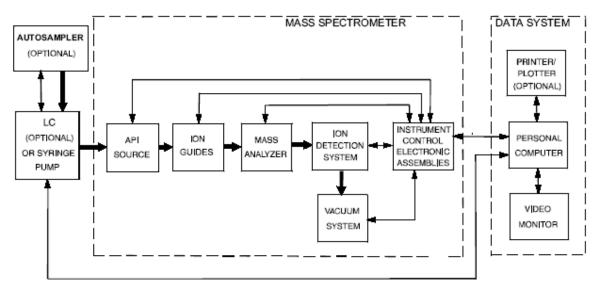
The ion, X+, will travel through the mass spectrometer just like any other positive ion - and will produce a line on the stick diagram.

All sorts of fragmentations of the original molecular ion are possible - and that means that you will get a whole host of lines in the mass spectrum.

Reaction whereby an ion is converted to another chemical structure or is broken down into ions with smaller masses.

## Finnigan TSQ Quantum

## Mass Spectrometer



Functional block diagram of the TSQ Quantum mass spectrometer. The broad, single-headed arrows represent the flow of sample molecules through the instrument. The narrow, double-headed arrows represent electrical connections.

## **Ion Polarity Modes**

The ion polarity mode of choice is determined by the polarity of the preformed ions in solution: Acidic molecules form negative ions in solution, and basic molecules form positive ions. The ejection of sample ions from droplets is facilitated if the ionic charge and surface charge of the droplet are of the same polarity. Thus, a positively charged needle is used to analyze positive ions and a negatively charged needle is used to analyze negative ions.

## Scan Modes

The scan modes in each category are as follows:

- Mass spectrometer scans modes: Q1MS and Q3MS
- MS/MS scan modes: Product, Parent and Neutral Loss
- Data dependent scan mode: Full scan, Selected ion monitoring (SIM), Selected reaction monitoring (SRM)

Scan Mode	Q1 Hyperquad	Q2 Collision Cell	Q3 Hyperquad
Q1MS	Scan <sup>a</sup>	Pass all ions <sup>b</sup>	Pass all ions
Q3MS	Pass all ions	Pass all ions	Scan
Product	Set <sup>d</sup>	Fragment ions <sup>e</sup> , then pass all fragments	Scan
Parent	Scan	Fragment ions, then pass all fragments	Set
Neutral Loss	Scan	Fragment ions, then pass all fragments	Scan

Table 1-1. Functions of the rod assemblies in different scan modes

aScan = full scan or transmission of selected ions

Pass all ions or fragments = pass ions or fragments within a wide range of mass-to-charge ratios

Fragment ions = collisions with argon gas cause ions to fragment

<sup>d</sup>Set = set to pass ions of a single mass-to-charge ratio or a set of mass-to-charge ratios

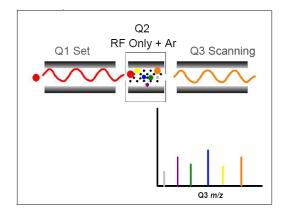
#### Page 18 of 45

#### Q1MS and Q3MS Scan Modes:

In the one stage of analysis, ions formed in the ion source enter the analyzer assembly. One of the mass analyzers (Q1 or Q3) is scanned to obtain a complete mass spectrum. The other rod assemblies (Q2 and Q3, or Q1 and Q2, respectively) act as ion transmission devices. In the Q1MS scan mode, Q1 is used as the mass analyzer; in the Q3MS scan mode, Q3 is used as the mass analyzer.

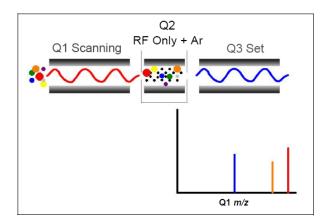
#### **Product (Daughter) Scan Mode:**

Two stages of analysis are performed. In the first stage, ions formed in the ion source enter Q1, which is set to transmit ions of one mass-to-charge ratio. Ions selected by this first stage of mass analysis are called *parent ions*. Parent ions selected by Q1 then enter Q2, which is surrounded by the collision cell. Collision cell can fragment further to produce *product ions by* collision-induced dissociation. Ions formed in the collision cell enter Q3 for the second stage of mass analysis. Q3 is scanned to obtain a mass spectrum.



#### Parent (Precursor) Scan Mode:

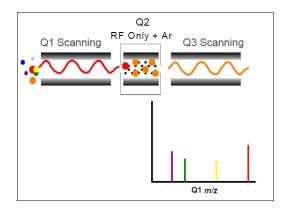
Two stage of mass analysis. In the first stage, ions formed in the ion source are introduced into the Parent mass analyzer (Q1), which is scanned to transmit parent ions sequentially into the collision cell. In the second stage of analysis, in the collision cell, parent ions can fragment to produce product ions by collision-induced dissociation Ions formed in the collision cell enter the Product mass analyzer (Q3), which transmits a selected product ion.



#### **Neutral Loss Scan Mode:**

Two stage of mass analysis. In the first stage, ions formed in the ion source are separated by mass-to-charge ratio by the Parent mass analyzer (Q1) and are introduced sequentially into the collision cell. In the second stage of analysis, ions admitted to the collision cell can fragment further to produce product ions by CID. These product ions are then separated by mass-to-charge ratio by the Product mass analyzer (Q3).

For an ion to be detected, between the time the ion leaves Q1 and enters Q3, it must lose a neutral moiety whose mass (the *Neutral Loss mass*) is equal to the difference in the mass ranges being scanned by the two mass analyzers. Thus, a spectrum is obtained (a *Neutral Loss mass spectrum*) that shows all the parent ions that lose a neutral species of a selected mass.



## Scan Types

TSQ Quantum Discovery systems can be operated with a variety of scan types. The most common scan types are as follows:

- Full scan
- Selected ion monitoring (SIM)
- Selected reaction monitoring (SRM)

#### Full Scan:

The *full scan scan type* provides a full mass spectrum of each analyte. With full scan, the scanning mass analyzer is scanned from the first mass to the last mass, without interruption, in a given scan time.

#### **Selected Ion Monitoring:**

*Selected ion monitoring (SIM)* is a technique in which a particular ion or set of ions is monitored. SIM experiments are useful in detecting small quantities of a target compound in a complex mixture when the mass spectrum of the target compound is known. SIM can improve the detection limit and decrease analysis time, but it can also reduce specificity.

#### **Selected Reaction Monitoring:**

In *selected reaction monitoring (SRM)*, a particular reaction or set of reactions, such as the fragmentation of an ion or the loss of a neutral moiety, is monitored. In SRM, a limited number of parent / product-ion pairs are monitored. In Product-type experiments, a parent ion is selected as usual, but generally only one product ion is monitored. SRM experiments are normally conducted with the Product scan mode. Any interfering compound would not only have to form an ion source product (parent ion) of the same mass-to-charge ratio as the selected parent ion from the target compound, but that parent ion would also have to fragment to form a product ion of the same mass-to-charge ratio as the selected product ion from the target compound.

## Data Types

You can acquire and display mass spectral data (intensity versus mass-to-charge ratio) with the TSQ Quantum Ultra mass spectrometer in one of two data types:

- Profile data type
- Centroid data type

#### **Profile Data Type:**

In the profile data type, you can see the shape of the peaks in the mass spectrum. Each atomic mass unit is divided into many sampling intervals. The intensity of the ion current is determined at each of the sampling intervals. The intensity at each sampling interval is displayed with the intensities connected by a continuous line. In general, the profile scan data type is used when you tune and calibrate the mass spectrometer so that you can easily see and measure mass resolution.

#### **Centroid Data Type:**

In the centroid data type, the mass spectrum is displayed as a bar graph. In this scan data type, the intensities of each set of multiple sampling intervals are summed. This sum is displayed versus the integral center of mass of the sampling intervals. In general, the centroid scan data type is used for data acquisition because the scan speed is faster and the disk space requirements are smaller. Data processing is also much faster for centroid data.

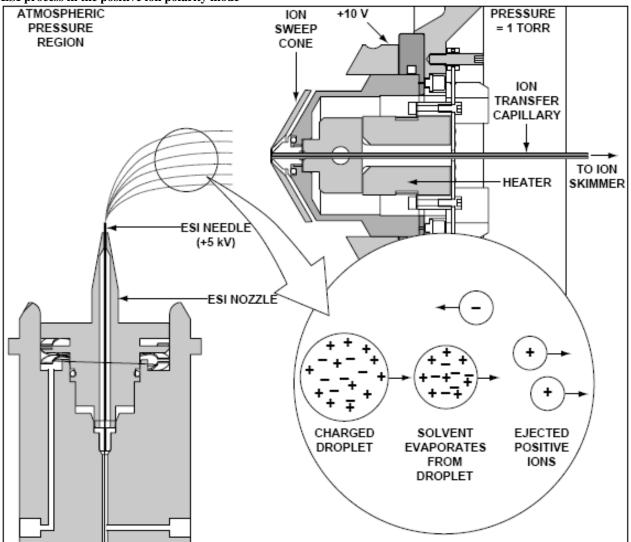
## **Electrospray Ionization** (Finnigan TSQ Quantum Discovery)

The *electrospray ionization (ESI) mode* transforms ions in solution into ions in the gas phase.

In ESI, ions are produced and analyzed as follows:

- 1. The sample solution enters the ESI needle, to which a high voltage is applied.
- 2. The ESI needle sprays the sample solution into a fine mist of droplets that are electrically charged at their surface.
- 3. The electrical charge density at the surface of the droplets increases as solvent evaporates from the droplets.
- 4. The electrical charge density at the surface of the droplets increases to a critical point known as the Rayleigh stability limit. At this critical point, the droplets divide into smaller droplets because the electrostatic repulsion is greater than the surface tension. The process is repeated many times to form very small droplets.
- 5. From the very small, highly charged droplets, sample ions are ejected into the gas phase by electrostatic repulsion.
- 6. The sample ions enter the mass spectrometer and are analyzed.

#### ESI process in the positive ion polarity mode



Organic solvents such as methanol, acetonitrile, and isopropyl alcohol are superior to water for ESI. Volatile acids and bases are good, but salts above 10 mM concentration and strong acids and bases are extremely detrimental. **The rules for achieving a good electrospray are:** 

- Keep salts out of the solvent system
- Use organic/aqueous solvent systems and volatile acids and bases
- Optimize the pH of the solvent system.

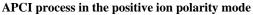
## Atmospheric Pressure Chemical Ionization (Finnigan TSQ Quantum Discovery)

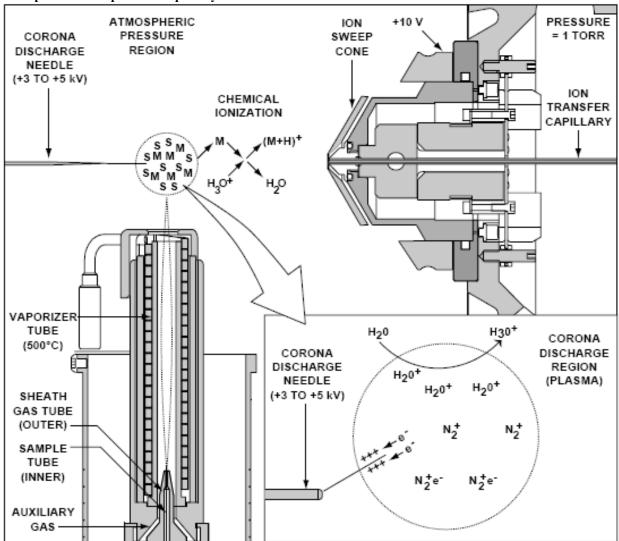
Atmospheric pressure chemical ionization (APCI) is a soft ionization technique, but not as soft as ESI. APCI is used to analyze compounds of medium polarity that have some volatility.

In APCI, ions are produced and analyzed as follows:

- 1. The APCI nozzle sprays the sample solution into a fine mist of droplets.
- 2. The droplets are vaporized in a high temperature tube (the vaporizer).
- 3. A high voltage is applied to a needle located near the exit end of the tube. The high voltage creates a corona discharge that forms reagent ions through a series of chemical reactions with solvent molecules and nitrogen sheath gas.
- 4. The reagent ions react with sample molecules to form sample ions.
- 5. The sample ions enter the mass spectrometer and are analyzed.

APCI is a gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process.





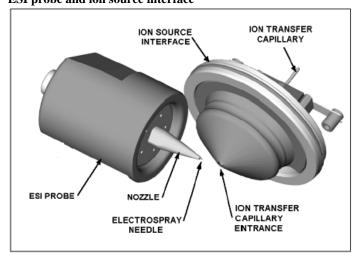
## API Probe (Finnigan TSQ Quantum Discovery)

The *API probe* is the source of sample ionization. You need to switch probes when you change ionization modes. Two API probes are available with the TSQ Quantum Discovery:

- ESI probe
- APCI probe

#### **ESI Probe:**

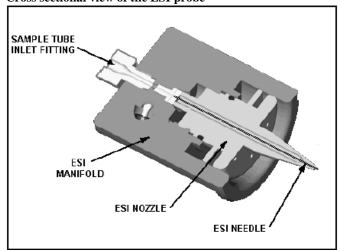
The *ESI probe* produces charged aerosol droplets that contain sample ions. See Figure. The ESI probe accommodates liquid flows of 1  $\mu$ L/min to 1 mL/min without splitting. **ESI probe and ion source interface** 



The ESI probe includes the ESI sample tube, needle, nozzle, and manifold. See Figure Sample and solvent enter the ESI probe through the sample tube. The *sample tube* is a short section of 0.1 mm ID fused-silica tubing that extends from a fitting secured to the ESI source housing, through the ESI probe and into the ESI needle, to within 1 mm from the end of the ESI needle. The ESI *needle*, to which a large negative or positive voltage is applied (typically  $\pm 3$  to  $\pm 5$  kV), sprays the sample solution into a fine mist of charged droplets. The ESI *nozzle* directs the flow of sheath gas and auxiliary gas at the droplets. The ESI *manifold* houses the ESI nozzle and needle and includes the sheath gas and auxiliary gas plumbing. The *sheath gas plumbing* and *auxiliary gas plumbing* deliver dry nitrogen gas to the nozzle.

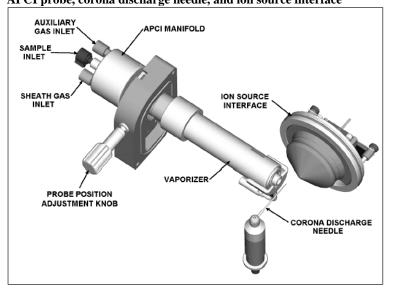
The ESI probe has inlets for the introduction of sample solution, sheath gas, and auxiliary gas into the API source. The *sheath gas* is the inner coaxial nitrogen gas that sprays (nebulizes) the sample solution into a fine mist as it exits the sample tube. Typical sheath gas flow rates for ESI are 10 to 30 units for sample flow rates of less than 10  $\mu$ L/min, and 30 to 60 units for sample flow rates greater than 400  $\mu$ L/min. When you tune the TSQ Quantum Discovery, you should adjust the sheath gas flow rate until the ion signal is stable.

The *auxiliary gas* is the outer coaxial nitrogen gas that assists the sheath gas in the nebulization and evaporation of sample solutions. The auxiliary gas also helps lower the humidity in the ion source. Typical auxiliary gas flow rates for ESI and APCI are 10 to 20 units. Auxiliary gas is usually not needed for sample flow rates below 50  $\mu$ L/min. **Cross sectional view of the ESI probe** 



#### **APCI Probe:**

The *APCI probe* ionizes the sample by atmospheric pressure chemical ionization. The APCI probe accommodates liquid flows of 100  $\mu$ L/min to 2 mL/min without splitting. See Figure 2-11. The APCI probe includes the APCI sample tube, nozzle, sheath gas and auxiliary gas plumbing, and vaporizer. See Figure 2-12. Sample and solvent enter the APCI nozzle through the *sample tube*. The sample tube is a short section of 0.15 mm ID fused silica tubing that extends from the sample inlet to 1 mm past the end of the nozzle. The *manifold* houses the APCI nozzle and includes the sheath gas and auxiliary gas plumbing. The *APCI nozzle* sprays the sample solution into a fine mist. The *sheath gas and auxiliary gas plumbing* deliver dry nitrogen gas to the nozzle. The droplets in the mist then enter the vaporizer. The *vaporizer* flash vaporizes the droplets at temperatures up to 600 °C. APCI probe, corona discharge needle, and ion source interface

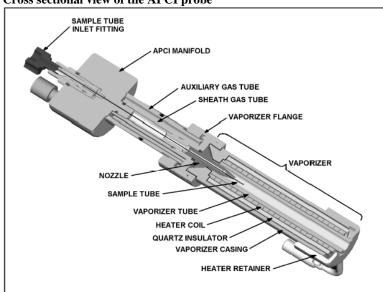


Typical vaporizer temperatures are 350 to 400 °C for flow rates of 100  $\mu$ L/min, 450 to 500 °C for 1 mL/min (normal APCI flow rate), and 550 to 600 °C for 2 mL/min. The sample vapor is swept toward the corona discharge needle by the flow of the sheath and auxiliary gasses.

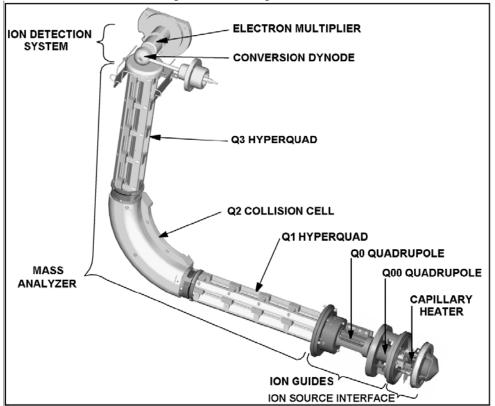
The *corona discharge needle assembly* is mounted inside of the APCI source housing. The tip of the corona discharge needle is positioned near the vaporizer. A high potential (typically  $\pm 3$  to  $\pm 5$  kV) is applied to the *corona discharge needle* to produce a *corona discharge current* of up to 10  $\mu$ A. (A typical value of the corona discharge current is 5  $\mu$ A.) The corona discharge from the needle produces reagent ion plasma primarily from the solvent vapor. The sample vapor is ionized by ion-molecule reactions with the reagent ions in the plasma.

APCI requires a constant source of electrons for the ionization process. Thus, the corona discharge current is set to a specific value and regulated. The potential applied to the corona discharge needle varies, as needed, to provide the required current.

Cross sectional view of the APCI probe



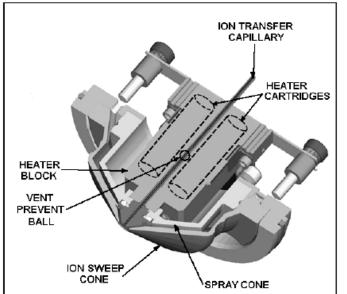
#### Ion Source Interface (Finnigan TSQ Quantum Discovery):



#### Internal (under vacuum) mass spectrometer components

The ion source interface includes an ion transfer capillary, two cartridge heaters, heater block, platinum probe sensor, vent prevent ball, and ion sweep cone.

- The ion transfer capillary assists in desolvating ions that are produced by the ESI or APCI probe.
- The *heater block* surrounds the ion transfer capillary and heats it to temperatures up to 400 °C.
- A *platinum probe sensor* measures the temperature of the heater block.
- The *vent prevent ball* falls into the space occupied by the ion transfer capillary when the capillary is removed, thus preventing air from entering the vacuum manifold. The vent prevent ball allows you to remove the ion transfer capillary for cleaning without venting the system.
- The *ion sweep cone* is a metallic cone over the capillary. The ion sweep cone acts as a physical barrier that protects the entrance of the capillary.
- The ion source interface is enclosed in a vacuum chamber that is evacuated by the rotary-vane pump to a pressure of approximately 1 Torr.



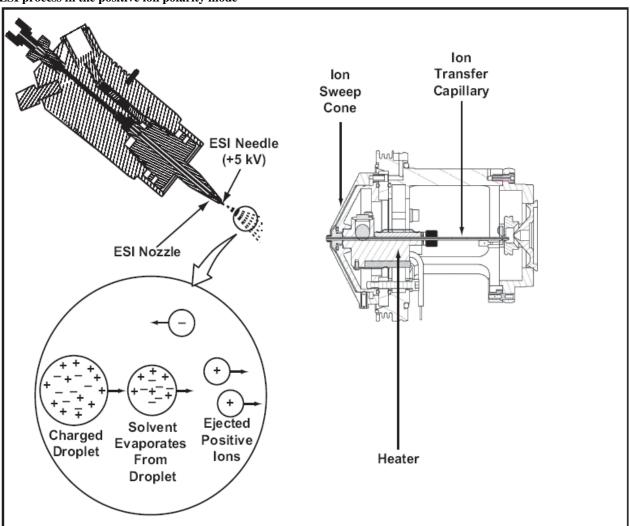
## Electrospray Ionization (Finnigan TSQ Quantum Ultra)

The *electrospray ionization (ESI) mode* transforms ions in solution into ions in the gas phase.1 Many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular weight compounds) can be analyzed by the use of ESI. ESI can be used to analyze any polar compound that makes a preformed ion in solution. The term *preformed ion* can include adduct ions. For example, polyethylene glycols can be analyzed from a solution containing ammonium acetate because of adduct formation between the NH4 <sup>+</sup> ions in the solution and oxygen atoms in the polymer. With ESI, the range of molecular weights that can be analyzed by the mass spectrometer is greater than 100,000 u, due to multiple charging. ESI is especially useful for the mass analysis of polar compounds, which include biological polymers (for example, proteins, peptides, glycoproteins, and nucleotides), pharmaceuticals and their metabolites, and industrial polymers (for example, polyethylene glycols).

In ESI, ions are produced and analyzed as follows:

- 1. The sample solution enters the ESI needle, to which a high voltage is applied.
- 2. The ESI needle sprays the sample solution into a fine mist of droplets that are electrically charged at their surface.
- 3. The electrical charge density at the surface of the droplets increases as solvent evaporates from the droplets.
- 4. The electrical charge density at the surface of the droplets increases to a critical point known as the Rayleigh stability limit. At this critical point, the droplets divide into smaller droplets because the electrostatic repulsion is greater than the surface tension. The process is repeated many times to form very small droplets.
- 5. From the very small, highly charged droplets, sample ions are ejected into the gas phase by electrostatic repulsion.
- 6. The sample ions enter the mass spectrometer and are analyzed.
- Figure shows the steps in the formation of ions from highly charged droplets.

ESI process in the positive ion polarity mode



You can use the ESI mode in either positive or negative ion polarity mode. The ion polarity mode of choice is determined by the polarity of the preformed ions in solution: acidic molecules form negative ions in solution, and

basic molecules form positive ions. The ejection of sample ions from droplets is facilitated if the ionic charge and surface charge of the droplet are of the same polarity. Thus, a positively charged needle is used to analyze positive ions and a negatively charged needle is used to analyze negative ions. Sample ions can carry a single charge or multiple charges. The number of

charges carried by the sample ion depends on the structure of the analyte of interest and the carrier solvent. (In ESI, the buffer and the buffer strength both have a noticeable effect on sensitivity. Therefore, it is important to proteins or peptides, the resulting mass spectrum consists typically of a series of peaks corresponding to a distribution of multiply charged analyte ions. The ESI process is affected by droplet size, surface charge, liquid surface tension, solvent volatility, and ion solvation strength. Large droplets with high surface tension, low volatility, strong ion solvation, low surface charge, and high conductivity prevent good electrospray. Organic solvents such as methanol, acetonitrile, and isopropyl alcohol are superior to water for ESI. Volatile acids and bases are good, but salts above 10 mM concentration and strong acids and bases are extremely detrimental.

The rules for achieving a good electrospray are:

• Keep salts out of the solvent system

• Use organic/aqueous solvent systems and volatile acids and bases

• Optimize the pH of the solvent system.

## Atmospheric Pressure Chemical Ionization (Finnigan TSQ Quantum Ultra)

Atmospheric pressure chemical ionization (APCI) is a soft ionization technique, but not as soft as ESI. APCI is used to analyze compounds of medium polarity that have some volatility.

In APCI, ions are produced and analyzed as follows:

1. The APCI nozzle sprays the sample solution into a fine mist of droplets.

2. The droplets are vaporized in a high temperature tube (the vaporizer).

3. A high voltage is applied to a needle located near the exit end of the tube. The high voltage creates a corona discharge that forms reagent ions through a series of chemical reactions with solvent molecules and nitrogen sheath gas.

4. The reagent ions react with sample molecules to form sample ions.

5. The sample ions enter the mass spectrometer and are analyzed.

Figure shows the APCI process for a positive adduct ion formation.

APCI is a gas phase ionization technique. Therefore, the gas phase acidities and basicities of the analyte and solvent vapor play an important role in the APCI process. In the positive-ion mode, sample ionization occurs in a series of reactions that start with the electron-initiated cation formation. Typical examples of primary, secondary, and adduct ion formation are shown below:

Primary ion formation

 $e^{-} + N_2 \rightarrow N_2^{+} + 2e^{-}$ 

Secondary ion formation

 $N_2^{+} + H_2O \rightarrow N_2 + H_2O^{+}$ 

 $H_2O^+ + H_2O \rightarrow H_3O^+ + HO^-$ 

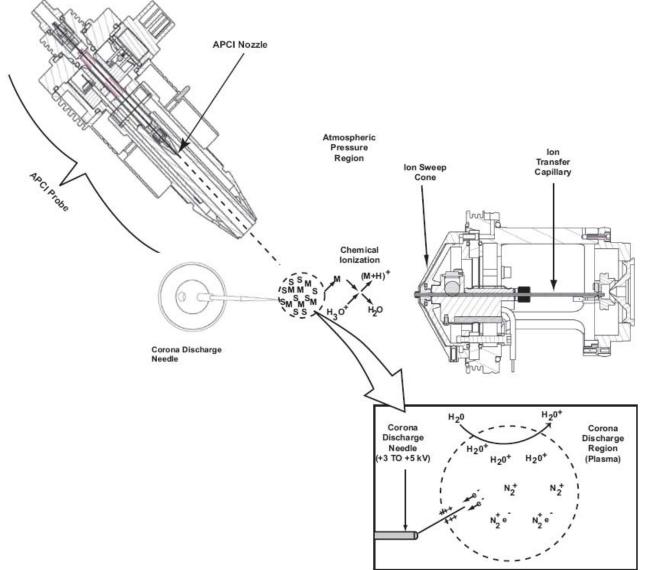
Proton transfer

 $\mathrm{H_3O^+} + \mathrm{M} \rightarrow \mathrm{(M + H)^+} + \mathrm{H_2O}$ 

In negative-ion mode,  $(M - H)^{-}$  is typically formed by the abstraction of a proton by OH<sup>-</sup>.

APCI is typically used to analyze small molecules with molecular weights up to about 1500 u. APCI is a very robust ionization technique. It is not affected by minor changes in most variables, such as changes in buffers or buffer strength.

#### APCI process in the positive ion polarity mode



You can use APCI in positive or negative ion polarity mode. For most molecules, the positive-ion mode produces a stronger ion current. This is especially true for molecules with one or more basic nitrogen (or other basic) atoms. An exception to the general rule is that molecules with acidic sites, such as carboxylic acids and acid alcohols, produce more negative ions than positive ions. Although, in general, fewer negative ions are produced than positive ions, negative ion polarity is sometimes the mode of choice. This is because the negative ion polarity mode sometimes generates less chemical noise than does the positive mode. Thus, selectivity might be better in the negative ion mode than in the positive ion mode.

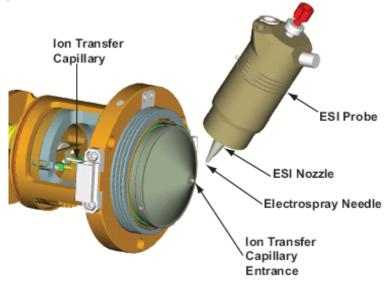
## API Source (Finnigan TSQ Quantum Ultra)

The *atmospheric pressure ionization (API) source* forms gas phase sample ions from sample molecules that are contained in solution. The API source also serves as the sample interface between the LC and the mass spectrometer. You can operate the API source in either the heated-electrospray ionization (H-ESI) or atmospheric pressure chemical ionization (APCI) mode.

#### **ESI** probe

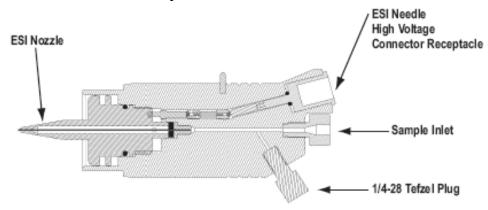
The *ESI probe* produces charged aerosol droplets that contain sample ions. See Figure the ESI probe accommodates liquid flows of 1  $\mu$ L/min to 1 mL/min without splitting.

#### ESI probe and ion source interface



The ESI probe includes the ESI sample tube, needle, nozzle, and manifold. See Figure Sample and solvent enter the ESI probe through the sample tube. The *sample tube* is a short section of 0.1 mm ID fused-silica tubing that extends from a fitting secured to the ESI source housing, through the ESI probe and into the ESI needle, to within 1 mm from the end of the ESI needle. The ESI *needle*, to which a large negative or positive voltage is applied (typically  $\pm 3$  to  $\pm 5$  kV), sprays the sample solution into a fine mist of charged droplets. The ESI *nozzle* directs the flow of sheath gas and auxiliary gas at the droplets. The ESI *manifold* houses the ESI nozzle and needle and includes the sheath gas and auxiliary gas plumbing. The *sheath gas plumbing* and *auxiliary gas plumbing* deliver dry nitrogen gas to the nozzle. The ESI probe has inlets for the introduction of sample solution, sheath gas, and auxiliary gas into the API source. The sheath gas flow rates for ESI are 10 to 30 units for sample flow rates of less than 10 µL/min, and 30 to 60 units for sample flow rates greater than 400 µL/min. When you tune the mass spectrometer, you should adjust the sheath gas flow rate until the ion signal is stable.

The *auxiliary gas* is the outer coaxial nitrogen gas that assists the sheath gas in the nebulization and evaporation of sample solutions. The auxiliary gas also helps lower the humidity in the ion source. Typical auxiliary gas flow rates for ESI and APCI are 10 to 20 units. Auxiliary gas is usually not needed for sample flow rates below 50  $\mu$ L/min.



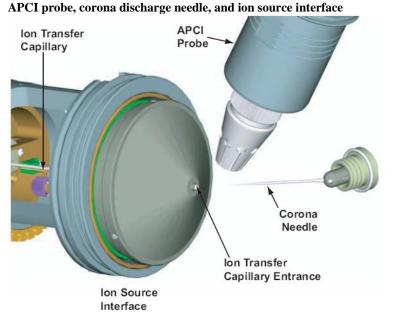
#### Cross sectional view of the ESI probe

The angle of the ESI probe is fixed at approximately sixty degrees. Adjustment screws allow you to make small changes to probe orientation to help optimize spray stability. The fixed angle off-axis spraying affords long-term signal stability (robustness) for most solutions containing non-volatile matrix components, mobile phase buffers, or ion-pairing reagents.

#### **APCI** probe

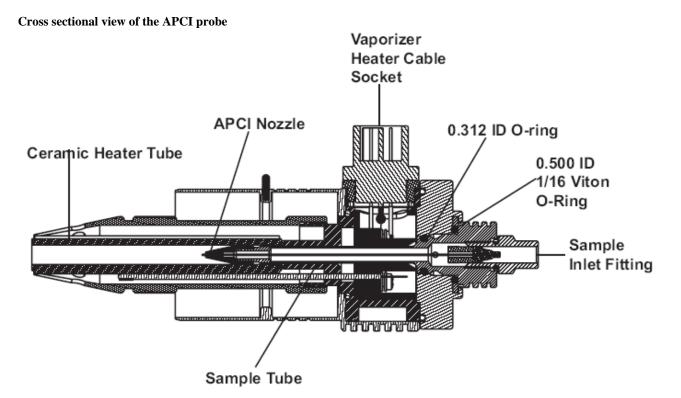
The *APCI probe* ionizes the sample by atmospheric pressure chemical ionization. The APCI probe accommodates liquid flows of 100  $\mu$ L/min to 2 mL/min without splitting. See Figure the APCI probe includes the APCI sample tube,

nozzle, sheath gas and auxiliary gas plumbing, and vaporizer. See Figure Sample and solvent enter the APCI nozzle through the *sample tube*. The sample tube is a short section of 0.10 mm ID fused silica tubing that extends from the sample inlet to 1 mm past the end of the nozzle. The *manifold* houses the APCI nozzle and includes the sheath gas and auxiliary gas plumbing. The *APCI nozzle* sprays the sample solution into a fine mist. The *sheath gas and auxiliary gas plumbing* deliver dry nitrogen gas to the nozzle. The droplets in the mist then enter the vaporizer. The *vaporizer* flash vaporizes the droplets at temperatures up to 500 °C.



Typical vaporizer temperatures are 350 °C to 450 °C for flow rates of 0.1 to 2 mL/min. The sample vapor is swept toward the corona discharge needle by the flow of the sheath and auxiliary gasses.

The corona discharge needle assembly is mounted inside the Ion Max API source housing. The tip of the corona discharge needle is positioned near the vaporizer. A high potential (typically  $\pm 3$  to  $\pm 5$  kV) is applied to the corona discharge needle to produce a corona discharge current of up to 100  $\mu$ A. (A typical value of the corona discharge current is 5  $\mu$ A.) The corona discharge from the needle produces reagent ion plasma primarily from the solvent vapor. The sample vapor is ionized by ion-molecule reactions with the reagent ions in the plasma. APCI requires a constant source of electrons for the ionization process. Thus, the corona discharge current is set to a specific value and regulated. The potential applied to the corona discharge needle varies, as needed, to provide the required current.



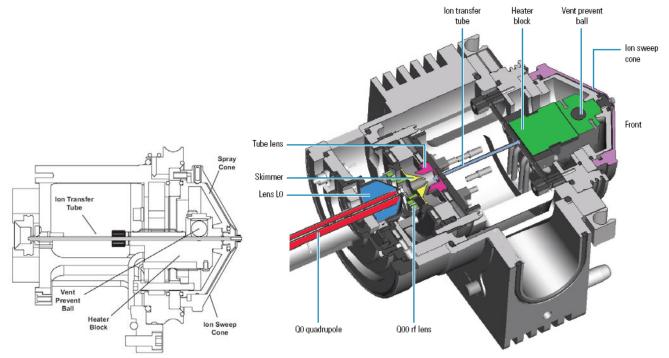
#### Ion Source Interface (Finnigan TSQ Quantum Ultra):

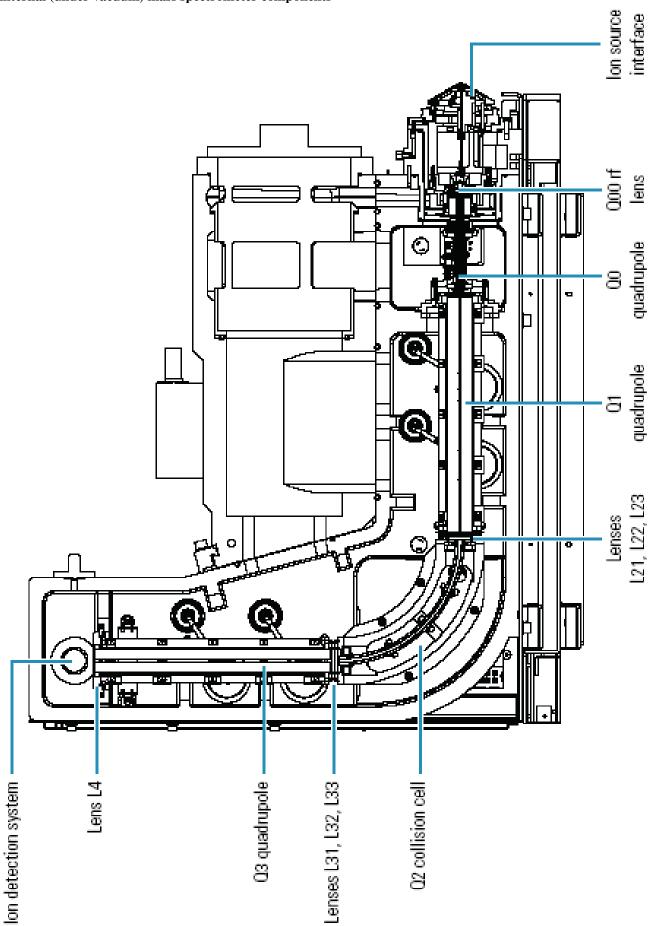
The *ion source interface* consists of the components of the API source that are held under vacuum (except for the atmospheric pressure side of the ion sweep cone). The ion source interface includes an ion transfer tube, two cartridge heaters, heater block, platinum probe sensor, vent prevent ball, and ion sweep cone See Figure

The *ion transfer tube* assists in desolvating ions that are produced by the H-ESI or APCI probe. The tube is an elongated, 4-in. cylindrical metal tube. Two *heater cartridges* are embedded in the heater block. The *heater block* surrounds the ion transfer tube and heats it to temperatures up to 400 °C. A *platinum probe sensor* measures the temperature of the heater

block. Typical temperatures of the ion transfer tube are 270 °C for H-ESI and 250 °C for APCI, but they will vary with flow rate and mobile phase composition. Ions are drawn into the ion transfer tube in the atmospheric pressure region and transported to the ion transfer tube-skimmer region of the vacuum manifold by a decreasing pressure gradient. A potential of typically  $\pm 35$  V (positive for positive ions and negative for negative ions) assists in repelling ions from the ion transfer tube to the skimmer. The *vent prevent ball* falls into the space occupied by the ion transfer tube when the tube is removed, thus preventing air from entering the vacuum manifold. The vent prevent ball allows you to remove the ion transfer tube for cleaning without venting the system. The *ion sweep cone* is a metallic cone over the ion transfer tube. The ion sweep cone channels the sweep gas towards the entrance of the tube. The system electronics include a voltage monitor circuit and an overtemperature/undertemperature circuit to protect the heaters. The voltage monitoring circuit detects shorting failures. The overtemperature portion of the circuit is intended to function as a thermal limit switch to prevent the heater from turning on continuously above a preset temperature. The undertemperature feature identifies faults in the platinum probe sensor that would otherwise cause the heater to turn full on. The ion source interface is enclosed in a vacuum chamber that is evacuated by the forepump to a pressure of approximately 1.5 Torr.

#### Cross sectional view of the ion source interface





## 1. Ion Guides

The *ion guides* focus the ions produced in the API source and transmit them to the mass analyzer. The TSQ Quantum Discovery uses two ion guides:

A. Q00 ion guide B. Q0 ion guide

#### A) Q00 Ion Guide:

The Q00 ion guide includes the tube lens, skimmer, Q00 quadrupole, interstage disk, and lens L0.

Ions from the ion transfer capillary enter the *tube lens*. The tube lens has a mass dependent potential applied to it to focus the ions towards the opening of the skimmer. An additional potential of between 0 and  $\pm 250$  V (positive for positive ions and negative for negative ions), called the *tube lens offset voltage*, can be applied to the tube lens to accelerate the ions into background gas that is present in the capillary-skimmer region.

- The *skimmer* acts as a vacuum baffle between the higher pressure ion source interface region (at 1.5 Torr) and the lower pressure Q00 ion guide region (at 50 mTorr by turbomolecular pump) of the vacuum manifold.
- The *Q00 quadrupole* is a square array of square-profile rods that acts as an ion transmission device. An RF voltage that is applied to the rods gives rise to an electric field that guides the ions along the axis of the quadrupole. A dc voltage offset from ground applied to Q00—called the *Q00 offset voltage*. the offset voltage is negative for positive ions and positive for negative ions
- The tube lens and skimmer mount to the *interstage disk*.
- The *lens L0* is a metal cylinder with a small hole in one end through which the ion beam can pass. A potential of between 0 and  $\pm 3$  V (negative for positive ions and positive for negative ions) is applied to lens L0 to aid in ion transmission. Lens L0 also acts as a vacuum baffle between the Q00 and Q0 ion gauge chambers.

#### **B) Q0 Ion Guide:**

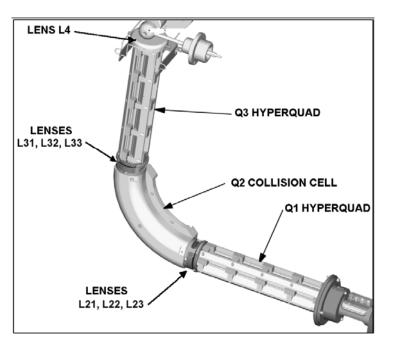
The *Q0 ion guide* transmits ions from the Q0 ion guide to the mass analyzer. The Q0 ion guide includes the Q0 quadrupole, lenses L11 and L12, mounting cage, baffle cap, and spring.

- The *Q0 quadrupole* is a square array of square-profile rods that acts as an ion transmission device similar to Q00. An RF voltage that is applied to the rods gives rise to an electric field that guides the ions along the axis of the quadrupole.
- The *L11* and *L12 lenses* are metal disks with a circular hole in the center through which the ion beam can pass. Together they act as a two-element cone lens. An electrical potential can be applied to the lens to accelerate (or decelerate) ions as they approach the lens and to focus the ion beam as it passes through the lens.

## 2. Mass Analyzer

The *mass analyzer* separates ions according to their mass-to-charge ratio and then passes them to the ion detection system. The mass analyzer on the TSQ consists of three quadrupole rod assemblies (Q1, Q2, and Q3) and three lens sets. The principal features of the mass analyzer and of mass analysis include the following:

- A. Quadrupole rod assemblies
- B. RF and dc fields applied to the quadrupoles
- C. Mass analysis
- D. Collision cell and CID efficiency
- E. Quadrupole offset voltage
- F. Mass analyzer lenses

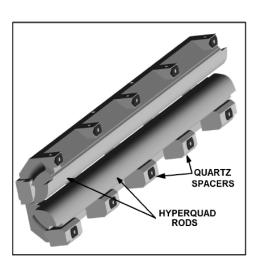


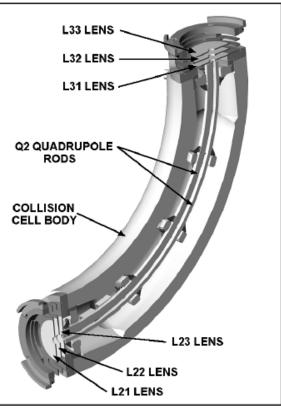
#### A) Quadrupole Rod Assemblies:

The TSQ Quantum Discovery mass analyzer has three rod assemblies. The first and third rod assemblies, Q1 and Q3, are  $HyperQuads^{TM}$  (hyperbolic-profile quadrupoles), and the second rod assembly, Q2, is a square-profile quadrupole.

The three rod assemblies used in the TSQ Quantum Discovery are numbered from the ion source end of the manifold and are designated Q1, Q2, and Q3. Q1 and Q3 are true hyperbolic quadrupoles—or "hyperquads"—that enable high-resolution scans without signal loss. The hyperquad rods are 250 mm long and the field radius (the distance from the surface of the rods to the z axis) is 6 mm. Quartz spacers act as electrical insulators between adjacent rods.

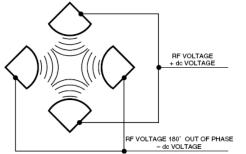
Q2 is a square-profile quadrupole rod assembly. Q2 always acts as an ion transmission device. The Q2 quadrupole rods are bent through a 90-degree arc. Q2 has become synonymous with the term *collision cell*. Technically, the collision cell is the chamber that encloses Q2 where collision-induced dissociation can take place if the argon collision gas is present.





#### **B) RF** and **DC** Fields Applied to the Quadrupoles:

In a quadrupole rod assembly, rods opposite each other in the array are connected electrically. Thus, the four rods can be considered to be two pairs of two rods each. Ac and dc voltages are applied to the rods and these voltages are ramped during the scan. Voltages of the same amplitude and sign are applied to the rods of each pair. However, the voltages applied to the different rod pairs are equal in amplitude but opposite in sign.



The ac voltage applied to the quadrupole rods is of constant frequency (1.123 MHz) and of variable amplitude (0 to 10,000 V peak-to-peak). Because the frequency of this ac voltage is in the radio frequency range, it is referred to as *RF voltage*. The dc voltage applied to the rods can vary from 0 to  $\pm$ 840 V. The ratio of RF voltage to dc voltage determines the ability of the mass spectrometer to separate ions of different mass-to-charge ratios. When both RF and dc voltages are applied, Q1 and Q3 function as mass analyzers. When only RF voltage is applied, they act as ion transmission devices.

The square quadrupole rod assembly (Q2) operates in the ion transmission mode only. Surrounding Q2 is a collision cell, the site where collision-induced dissociation (CID) can take place if the argon collision gas is present in the cell.

#### C) Mass Analysis:

The *mass analyzers* (Q1 and Q3) are square arrays of precision-machined and precision-aligned hyperbolic rods. The rods are charged with a variable ratio of RF voltage and dc voltage (Figure 2-21). These potentials give rise to an electrostatic field that gives stable oscillations to ions with a specific mass-to-charge ratio and unstable oscillations (These ions strike one of the rod surfaces or are ejected from the rod assembly, become neutralized, and are pumped away.) to all others.

The potentials on the quadrupole rods can be changed rapidly and precisely. The RF and dc voltages in the TSQ Quantum Discovery can be scanned over the full mass range of the system (for example, 30 to 1500 u) in as short a time as 0.1 s. (Although, under the conditions usually employed in mass analysis, such a scan would normally be done in about 2 s.)

At the end of the scan, the RF and dc voltages are discharged to zero,

#### D) Collision Cell and CID Efficiency:

The *collision cell quadrupole rod assembly* (Q2), which always acts as an ion transmission device, is a quadrupole array of square-profile rods. The rods are charged with a variable RF voltage. This RF voltage gives rise to an electrostatic field that gives stable oscillations to ions in a wide window of mass-to-charge ratios.

In the MS/MS scan modes, the collision cell is emptied of ions in between scans by applying a large voltage of opposite polarity to the rod pairs. This ensures that no ions remain in the collision cell from scan to scan.

Surrounding Q2 is the *collision cell*. The collision cell is usually pressurized from about 1 to  $4 \times 10_{-3}$  Torr with argon *collision gas*. The collision cell is the site where *collision-induced dissociation (CID)* takes place.

#### E) Quadrupole Offset Voltage:

The *quadrupole offset voltage* is a dc potential applied to the quadrupole rods in addition to the ramping dc voltage. The offset voltage applied to the two rod pairs of the assemblies are equal in amplitude and equal in sign. The purpose of the quadrupole offset voltage is to accelerate or decelerate ions and, thus, to set the translational kinetic energy (TKE) of the ions as they enter the quadrupole rod assembly.

In general, the offset voltages applied to Q1 and Q2 are fixed for a given experiment. However, in MS/MS experiments, the quadrupole offset voltage applied to Q3 usually varies as a scan proceeds. The TSQ Quantum Discovery automatically computes the Q3 quadrupole offset voltage necessary for a given experiment and then varies the voltage, as appropriate, as each scan proceeds.

The offset voltage applied to Q2 (which contains the collision cell) is called the *collision energy*. The collision energy is the difference in potential between the API source (where parent ions are formed) and Q2 (where they collide with collision gas).

Before any mass spectra are obtained, Q1 is tuned in the Q1MS scan mode (Q2 and Q3 RF voltage only), and Q3 is tuned in the Q3MS scan mode (Q1 and Q2 RF voltage only). During tuning, the optimum quadrupole offset voltage is determined for Q1 and for Q3.

#### F) Mass Analyzer Lenses

The TSQ Quantum Discovery mass analyzer has three lens sets. Those between Q1 and Q2 are designated L21, L22, L23; those between Q2 and Q3 are designated L31, L32, L33; and the lens between Q3 and the ion detection system is designated as L4 (or exit lens). All of the lenses have circular holes in their centers through which the ion beam passes.

The *L2x lens set* (between Q1 and Q2) and the *L3x lens set* (between Q2 and Q3) serve three functions. Their first function is to minimize the amount of collision gas that enters the mass analyzers (Q1 and Q3) from the collision cell (Q2).

The second function of the L2x and L3x lens sets is to shield Q1 from the RF voltage applied to Q2 and vice versa (L2x lens set) and to shield Q3 from the RF voltage applied to Q2 and vice versa (L3x lens set).

The third function of the L2x and L3x lens sets is to focus the ion beam. The three lenses between Q1 and Q2 (and those between Q2 and Q3) together form a three-element aperture lens. The first and third lenses are generally set to similar or identical values and the central lens is set to a value different (either higher or lower) from the other two. The voltage applied to each of the lenses can vary from about -300 to +300 V. the voltage applied to the first and third elements of the L2x lens set is somewhat greater than the quadrupole offset voltage applied.

*Lens L4* is located between Q3 and the ion detection system. L4 is held at ground potential. Its purpose is to shield Q3 from the high voltage applied to the ion detection system and to shield the ion detection system from the high RF voltages applied to Q3.

### **3.** Ion Detection System

The TSQ Quantum Discovery is equipped with a high sensitivity, off-axis *ion detection system* that produces a high signal-to-noise ratio and allows for voltage polarity switching between positive ion and negative ion modes of operation. The ion detection system includes a 15-kV conversion dynode and a channel electron multiplier. The ion detection system is located at the rear of the vacuum manifold behind the mass analyzer.

The conversion dynode is a concave metal surface that is located at a right angle to the ion beam. A potential of +15 kV for negative ion detection or -15 kV for positive ion detection is applied to the conversion dynode. When an ion strikes the surface of the conversion dynode, one or more secondary particles are produced. These secondary particles can include positive ions, negative ions, electrons, and neutrals. When positive ions strike a negatively charged conversion dynode, the secondary particles of interest are negative ions and electrons. When negative ions strike a positively charged conversion dynode, the secondary particles of interest are positive ions. These secondary particles are focused by the curved surface of the conversion dynode and are accelerated by a voltage gradient into the electron multiplier.

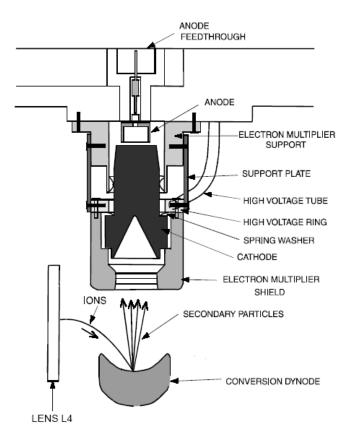
The electron multiplier includes a cathode and an anode. The cathode of the electron multiplier is a lead-oxide, funnellike resistor. A potential of up to -2.5 kV is applied to the cathode by the high voltage ring. The exit end of the cathode (at the anode) is near ground potential. The cathode is held in place by the high voltage ring, two support plates, the electron multiplier support, and the electron multiplier shield. A spring washer applies a force to the cathode to hold it in contact with the electron multiplier shield. The electron multiplier support is attached to a base plate that is mounted to the vacuum manifold by three screws.

The anode of the electron multiplier is a small cup located at the exit end of the cathode. The anode collects the electrons produced by the cathode. The anode screws into the anode feedthrough in the base plate.

Secondary particles from the conversion dynode strike the inner walls of the electron multiplier cathode with sufficient energy to eject electrons. The ejected electrons are accelerated farther into the cathode, drawn by the

increasingly positive potential gradient. Due to the funnel shape of the cathode, the ejected electrons do not travel far before they again strike the inner surface of the cathode, thereby causing the emission of more electrons. Thus, a cascade of electrons is created that finally results in a measurable current at the end of the cathode where the electrons are collected by the anode. The current collected by the anode is proportional to the number of secondary particles striking the cathode.

#### Cross sectional view of the ion detection system, showing the electron multiplier and the conversion dynode



#### How do set up the Mass Spectrometer for various lc flow rates in TSQ Quantum? Guidelines for setting operating parameters for LC/ESI/MS (spray voltage 3 to 4.5 kV)

LC Flow Rate (μL/min)	Suggested Column ID Size (mm)	Capillary Temperature (°C)	Sheath Gas (psi)	Auxiliary Gas (arbitrary units)
≤ 10	Capillary	200 to 250	5 to 30	Off
50 to 100	1.0	250 to 300	10 to 30	5 to 10
200 to 400	2.1 to 4.6	300 to 350	20 to 40	10 to 20
≥ 400	4.6	350	30 to 60	10 to 40

#### Guidelines for setting operating parameters for LC/H-ESI/MS (compound dependent)

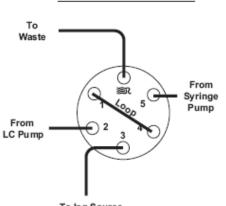
LC Flow Rate (µL/min)	Suggested Column ID Size (mm)	H-ESI Vaporizer Temperature (°C)	Spray Voltage (V)	Capillary Temperature (°C)	Sheath Gas (psi)	Auxiliary Gas (arbitrary units)
≤ 10	Capillary	0 (off) to 50	3000(-2500*)	200 to 250	5 to 30	Off
50 to 100	1.0	50 to 200	3000 (-2500)	250 to 300	10 to 30	5 to 10
200 to 400	2.1 to 4.6	200 to 400	3000 (-2500)	300 to 350	20 to 40	10 to 20
≥ 400	4.6	300 to 450	3000 (-2500)	350	30 to 60	10 to 40

#### Guidelines for setting operating parameters for LC/APCI/MS

LC Flow Rate	Capillary	APCI Vaporizer	Sheath Gas		Corona Discharge
(mL/min)	Temperature (°C)	Temperature (°C)	(psi)		Current (µA)
0.2 to 2.0	200 to 350	400 to 600	30 to 40	0 to 5	+4 (-10)

\*negative ion mode

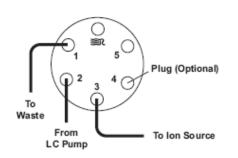
#### Divert/inject valve plumbed as a loop injector and as a divert valve

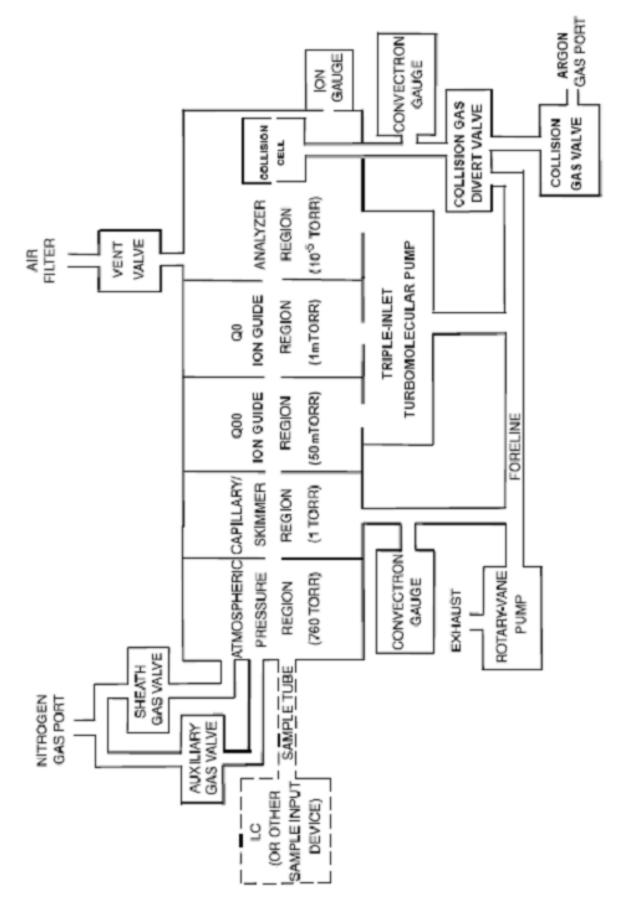


Plumbed as a Loop Injector

To lon Source

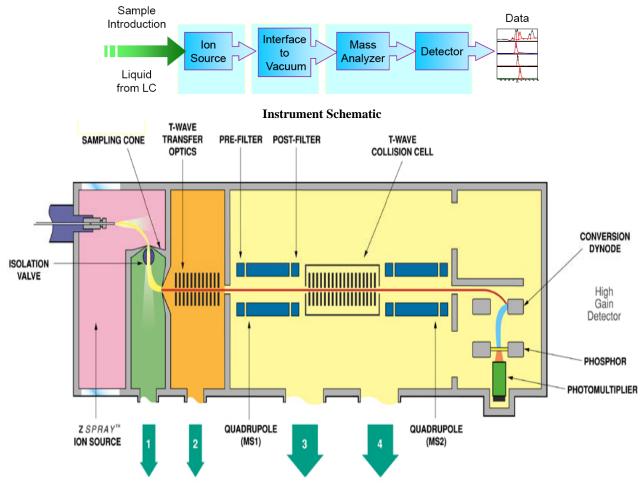
#### Plumbed as a Divert Valve





## Waters Quattro Premier

## Instrumentation



## 1. Ionization Techniques

Two atmospheric pressure ionization techniques are available:

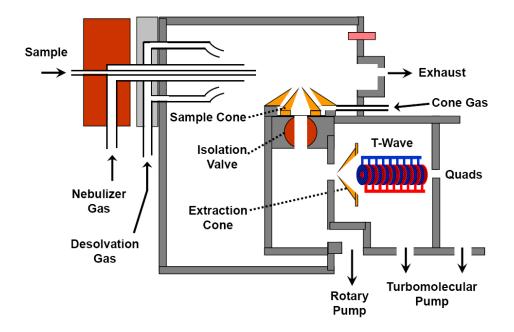
- Electrospray ionization.
- Atmospheric pressure chemical ionization.

## 1.1 Electrospray Ionization (ESI)

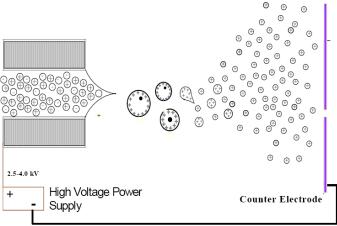
Electrospray ionization takes place as a result of imparting a strong electrical field to the eluent flow as it emerges from the nebulizer, producing an aerosol of charged droplets. These undergo a reduction in size by solvent evaporation until they have reached a charge density sufficient to allow sample ions to be ejected from the droplet's surface ("ion evaporation").

A characteristic of ESI spectra is that ions may be singly- or multiply-charged. Since the mass spectrometer filters ions according to their mass-to-charge ratio, compounds of high molecular weight can be determined if multiply-charged ions are formed. Eluent flows up to 1 mL/min can be accommodated, although it is often preferable to split the flow such that 100 to 200  $\mu$ L/min of eluent enters the mass spectrometer.

-This illustration shows the ZSpray Source for Electrospray Ionization (ESI).

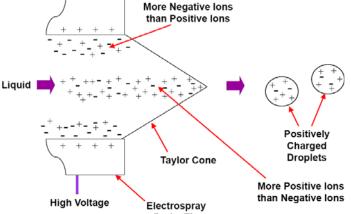


- $\rightarrow$  Liquid is sprayed from a capillary tube, to which a high voltage is applied.
- $\rightarrow$ A spray of charged droplets forms



 $\rightarrow$ when you apply a positive high voltage to the electrospray capillary, the droplets emitted from the capillary carry an excess of positive charge(that is, there are more cations than anions in the droplet). The excess charge in droplet is at or very near the droplet surface. The distance between charges is maximized, and therefore, charge repulsion is minimized when the charges are at surface. In contrast, the interior of the droplet is neutral, and contain solvent, other molecules, and ion-paired species. As the solvent evaporates, charge repulsion at the surface forces the droplet to break into several smaller droplets. The end result of the process leaves ions in the gas phase.

 $\rightarrow$  When you apply negative high voltage to the electrospray capillary, the droplets emitted from the capillary carry an excess of negative charge.

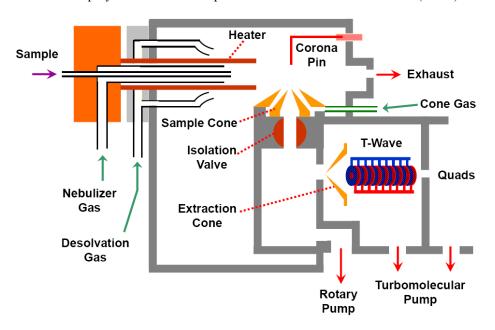


Probe Tip

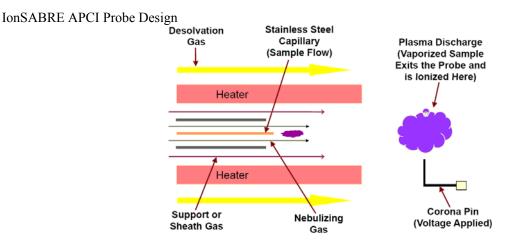
 $\rightarrow$ This illustration is expanded view of the capillary that shows a droplet formation during positive ion electrospray.

## 1.2 Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric pressure chemical ionization generally produces protonated or deprotonated molecular ions from the sample via a proton transfer (positive ions) or proton abstraction (negative ions) mechanism. The sample is vaporized in a heated nebulizer before emerging into a cloud of solvent ions formed within the atmospheric source by a corona discharge. Proton transfer, or abstraction, then takes place between the solvent ions and the sample. -This illustration shows the ZSpray Source for Atmospheric Pressure Chemical Ionization (APCI).



 $\rightarrow$  Liquid is passed through a heated tube (fused silica capillary), then evaporated to produce gas phase molecules.  $\rightarrow$ Applying high voltage to a corona pin, near the exit of the tube, produces a cloud of ionized nitrogen atoms that ionize the molecules as they pass through.

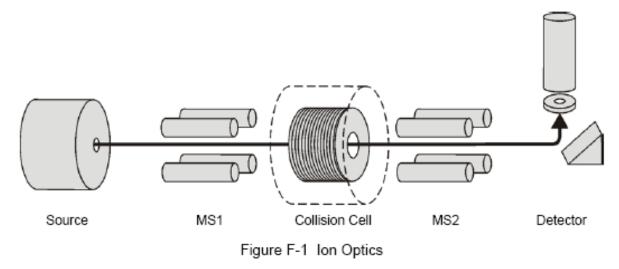


In this illustration, the sample flows through the capillary into the heater in a fine spray with the assistance of the nebulizing gas. The heater is set to rapidly vaporize the analytes and mobile phase. The nebulizer gas and sheath gas direct the vaporized sample out of the heater and toward the plasma discharge. The corona pin ionizes the nitrogen gas in a small region of the source, which is the plasma discharge.

- $\rightarrow$ Higher temperature, more aggressive ionization
- $\rightarrow$ Solvent molecules are in the gas phase
- $\rightarrow$ Ionization takes place in the plasma
- $\rightarrow$ Goal of the nitrogen is to evaporate solvent expelled from fused silica
- →Potentially more sensitive than electrospray with some non-polar molecules

## 2. Ion Optics

Figure F-1 shows the Quattro Premier XE ion optics.



## 3. MS Operating Modes

Table F-1 shows the MS operating modes.

Operating Mode	MS1	Collision Cell	MS2
MS1	Resolving (scanning)	Pass all masses	
MS2	Pass all masses		Resolving (scanning)
SIR	Resolving (static)	Pass all masses	

The MS1 mode, in which MS1 is used as the mass filter, is the most common and most sensitive method of performing MS analysis. This is directly analogous to using a single quadrupole mass spectrometer.

The MS2 mode of operation is used, with collision gas present, when switching rapidly between MS and MS/MS operation (for example, survey scan mode). It also provides a useful tool for instrument tuning and calibration before MS/MS analysis, and for fault diagnosis.

The SIR (Selected Ion Recording) mode of operation is used as a quantitation mode when no suitable fragment ion can be found to perform a more specific MRM analysis (see Section 4.3).

## 4. MS/MS Operating Modes

The four common MS/MS operating modes are summarized in Table F-2.

Table F_2	2M/2M	Operating	Modee
able 1-2	MONNO	Operating	woues

Operating Mode	MS1	Collision Cell	MS2
Daughter (Product) Ion Spectrum	Static (at parent mass)		Scanning
Parent (Precursor) Ion Spectrum	Scanning	Pass all	Static (at daughter mass)
Multiple Reaction Monitoring (MRM)	Static (at parent mass)	masses	Static (at daughter mass)
Constant Neutral Loss Spectrum			Scanning (synchronized with MS1)

## 4.1 Daughter (Product) Ion Mode

The daughter (product) ion mode is shown in Figure F-2. It is the most commonly used MS/MS operating mode.

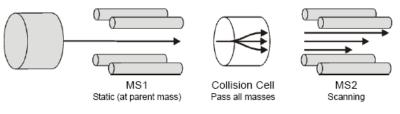


Figure F-2 Daughter (Product) Ion Mode

#### **Typical Applications**

• Structural elucidation (for example, peptide sequencing)

- Method development for MRM screening studies:
- Identification of daughter ions for use in MRM transitions.

– Optimization of CID tuning conditions to maximize the yield of a specific daughter ion to be used in MRM analysis. Figure F-3 shows an example of daughters of the specific parent at m/z 609 from reserpine in Electrospray positive ion mode.

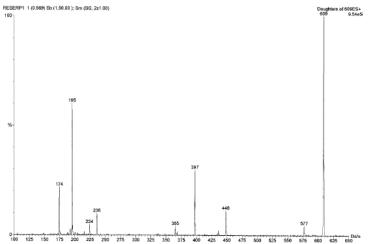


Figure F-3 Daughters of the Specific Parent at m/z 609 from Reserpine in Electrospray Positive lon Mode

## 4.2 Parent (Precursor) Ion Mode

The parent (precursor) ion mode is shown in Figure F-4.

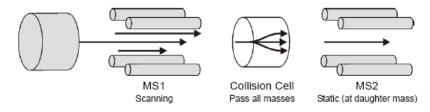
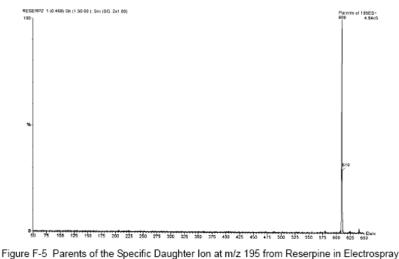


Figure F-4 Parent (Precursor) Ion Mode

A typical application is for structural elucidation, that is, complementary or confirmatory information (for daughter scan data).

Figure F-5 shows an example of parents of the specific daughter ion at m/z 195 from reserpine in electrospray positive ion mode.



Positive Ion Mode

## 4.3 Multiple Reaction Monitoring (MRM) Mode

The MRM mode (Figure F-6) is a highly selective MS/MS equivalent of SIR. As both MS1 and MS2 are static, greater dwell time on the ions of interest is allowed, and therefore better sensitivity compared to scanning MS/MS.

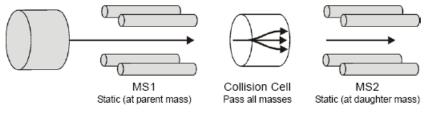


Figure F-6 MRM Mode

A typical application is for quantification of known analytes in complex samples:

- Drug metabolite and pharmacokinetic studies.
- Environmental, for example, pesticide and herbicide analysis.
- Forensic or toxicology, for example, screening for target drugs in sport.
- MRM does not produce a spectrum as only one transition is monitored. As in SIR, a chromatogram is produced.

### 4.4 Constant Neutral Loss Mode

The constant neutral loss mode is shown in Figure F-7. It detects the loss of a specific neutral fragment or functional group from an unspecified parent or parents.

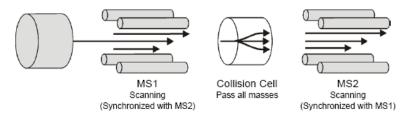


Figure F-7 Constant Neutral Loss Mode

A typical application is for screening mixtures for a specific class of compound that is characterized by a common fragmentation pathway, indicating the presence of compounds containing a common functional group. The scans of MS1 and MS2 are synchronized. When MS1 transmits a specific parent ion, MS2 "looks" to see if that

parent loses a fragment of a certain mass. If it does, it registers at the detector.

The result is that the spectrum shows the masses of all parents that actually lost a fragment of a certain mass.

## 4.5 Source and Collision Cell T-Wave Devices

The T-Wave devices are stacked ring electrode ion guides with opposite phases of radio frequency voltage applied to adjacent plates to confine ions radially (Figure F-8).

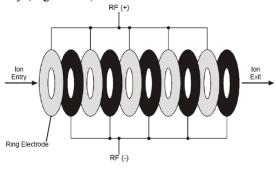


Figure F-8 T-Wave Schematic Diagram

The presence of gas in these devices causes an axial slowing of ions, which can have adverse effects for fast acquisitions such as reduced sensitivity resolution and increased cross-talk. To reduce the residence time of ions in these optics, a travelling voltage wave (T-Wave) moves along the device by application of a transient d.c. voltage to successive ring electrodes. Through appropriate choice of wave amplitude and velocity, ions "surf" on the front of this wave, reducing their transit time (Figure F-9).

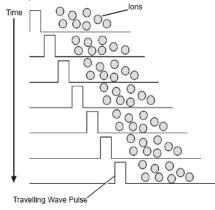


Figure F-9 Travelling Wave Pulse

Under normal operating conditions, the wave parameters are fixed by MassLynx to give optimal performance. However, under certain operating conditions, manual control may be necessary