Chapter 1
Introduction

The Thermo Finnigan LCQ™ Advantage is an advanced analytical instrument that includes a syringe pump, an optional divert/inject valve, an atmospheric pressure ionization (API) source, a mass spectrometer (MS) detector, and the Xcalibur® data system. In a typical analysis, a sample can be introduced in any of the following ways:

- Using the syringe pump (direct infusion)
- Using the inject valve fitted with a loop and an LC (flow injection analysis)
- Using a divert valve and HPLC fitted with a column (LC/MS)

In analysis by LC/MS, a sample is injected onto an LC column. The sample is then separated into its various components. The components elute from the LC column and pass into the MS detector where they are analyzed. Analysis by direct infusion or flow injection provides no chromatographic separation of components in the sample before it passes into the MS detector. The data from the MS detector are then stored and processed by the data system.

This introduction answers the following questions:

- Why use the LCQ Advantage MS detector?
- Which MS detector technique—ESI or APCI—is better for analyzing my samples?
- How can I introduce my samples into the MS detector?
- What types of buffers should I use? What types should I avoid?
- How should I set up the MS detector for various LC flow rates?
- What is calibrating and tuning the MS detector all about?
- What type of experiments can I perform with LCQ Advantage?

1.1 Why Use the LCQ Advantage MS Detector?

The attribute that sets the LCQ Advantage MS detector apart from other LC detectors is the high level of analytical specificity that it provides. The LCQ Advantage can provide five levels of analysis. Each level of analysis adds a new dimension of specificity for positive compound identification. The five levels of analysis are as follows:

- Chromatographic separation and compound detection (retention time)
- Mass analysis (molecular weight information)
- Two-stage mass analysis (structural information)
- Wideband Activation (structural information)
- ZoomScan analysis (higher resolution scan)

Chromatographic separation and compound detection are obtained by all LC/detector systems. Retention time alone, however, does not positively identify a compound because many compounds can have the same retention time under the same experimental conditions. In addition, even if a compound is identified correctly by retention time, quantitation results can be in error because other compounds in the sample might coelute with the compound of interest.

Mass analysis allows for the identification of analytes of interest. Atmospheric pressure ionization typically produces mass spectra that provide molecular weight information, either directly for relatively non-polar small molecules or after a mathematical manipulation for proteins or peptides.

Two-stage mass analysis allows for even more positive compound identification. MS/MS analysis monitors a reaction path: the production of a specific product ion from a specific parent ion [called selective reaction monitoring (SRM)]. Using SRM analysis, for example, you can easily quantitate target analytes in complex matrices such as plant or animal tissue, plasma, urine, groundwater, and soil. Because of the specificity of MS/MS measurements and the ability to eliminate interferences by an initial mass selection stage, quantitative target compound analysis is easily accomplished using the LCQ Advantage MS detector.

The Wideband Activation option adds energy, within a range of 20 u, during MS/MS fragmentation. This is useful when a loss of water or ammonia is observed. Choose this feature for qualitative MS/MS when you want enhanced structural information. Signal sensitivity is somewhat reduced when you choose this option.

ZoomScan analysis provides information about the charge state of one or more mass ions of interest. ZoomScan data is collected by using slower scans in a narrow range at higher resolution. This can improve the resolution of the ^{12}C / ^{13}C isotopes of the analyte ion, which allows for unambiguous determination of charge state, which in turn allows for the correct determination of molecular weight.

1.2 Which MS Detector Technique—ESI or APCI—Is Better for Analyzing My Samples?

You can operate the MS detector in either of two atmospheric pressure ionization modes:

- Electrospray ionization (ESI)
- Atmospheric pressure chemical ionization (APCI)

Typically, more polar compounds such as amines, peptides, and proteins are best analyzed by ESI, and non-polar compounds such as steroids are best analyzed by APCI.

Sample ions can carry a single charge or multiple charges. The number of charges carried by the sample ions depends on the structure of the analyte of interest and the mobile phase.

Using ESI/MS

The ***ESI*** mode typically produces mass spectra consisting of multiply charged ions (for proteins and peptides) depending on the structure of the analyte and the solvent. For example, the resulting mass spectrum of a higher molecular weight protein or peptide typically consists of a distribution of multiply charged analyte ions. The resulting mass spectrum can be mathematically manipulated to determine the molecular weight of the sample.

The ESI mode transfers ions in solution into the gas phase. Many samples that previously were not suitable for mass analysis (for example, heat-labile compounds or high molecular weight compounds) can be analyzed by 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 NH_4^+ ions in the solution and oxygen atoms in the polymer. With ESI, the range of molecular weights that can be analyzed by the LCQ Advantage 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).

You can use the ESI mode in either positive or negative ion polarity mode. The ion polarity mode is determined by the polarity of the preformed ions in solution: Acidic molecules form negative ions in high pH solution, and basic molecules form positive ions in low pH solution. A positively charged ESI needle is used to generate positive ions and a negatively charged needle is used to generate negative ions.

You can vary the flow rate from the LC into the MS detector over a range from 1 µL/min to 1000 µL/min. Refer to Table . (In ESI, the buffer and the buffer strength both have a noticeable effect on sensitivity. Therefore, it is important to choose these variables correctly.) In the case of higher molecular weight 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 and strong acids and bases are extremely detrimental.

The rules for a good electrospray are as follows:

- Keep salts and non-volatile buffers out of the solvent system. For example, avoid the use of salts containing sodium or potassium and avoid the use of phosphates.
- Use organic/aqueous solvent systems and volatile acids and bases.
- Optimize the pH of the solvent system for your analyte of interest. For example, if your analyte of interest is a compound containing carboxylic acid, your mobile phase should be slightly alkaline (pH 8 or 9) to keep the acid functional groups in solution as negative ions.

Using APCI/MS

Like ESI, *APCI* is a soft ionization technique. APCI provides molecular weight information for compounds of medium polarity that have some volatility.

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.

APCI is typically used to analyze small molecules with molecular weight up to about 2000 u. APCI is a very robust ionization technique. It is not affected by minor changes in most variables such as changes in buffer or buffer strength. The rate of solvent flowing from the LC into the MS detector in APCI mode is typically high (between 0.2 and 2 mL/min). See Table 1-3.

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. Molecules which generally produce strong negative ions, with acidic sites such as carboxylic acids and acid alcohols, are an exception to this general rule.

Although, in general, fewer negative ions are produced than positive ions, negative ion polarity can be more specific. 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.

1.3 Should I Use Auxiliary Gas or Sweep Gas?

Nitrogen gas can be applied to the system in one of two ways, as Auxiliary (AUX) gas or as Sweep gas. When AUX gas is being used, nitrogen flows through the ion source nozzle, the vapor plume is affected, the spray is focused, and desolvation is improved. When Sweep gas is used, the nitrogen flows out from behind the sweep cone and can result in solvent declustering and adduct reduction.

When you are analyzing complex matrices such as plasma or non-volatile salt buffers, Sweep gas is required for ruggedness. In full-scan MS or data dependent scan experiments, the signal-to-noise ratio can be improved by application of Sweep gas. In some cases, signal intensity can be increased by using AUX gas, particularly for higher LC flow rates.

All analyses are analyte dependent and require separate optimization with AUX gas and Sweep gas to determine which will yield optimum performance. It is especially important to optimize with each gas independently before you perform experiments using MS^n techniques and before you perform any quantitative analysis experiments because optimum results could be achieved with either AUX or Sweep gas. Refer to Table 1-2 and Table 1-3 for additional information on using AUX/Sweep gas.

1.4 How Can I Introduce My Samples into the MS Detector?

You can introduce your samples into the MS detector in a variety of ways. Refer to Table 1-1.

The syringe pump is often used to introduce calibration solution for automatic tuning and calibrating in ESI mode. You can also use this technique to introduce a solution of pure analyte at a steady rate in ESI mode, for example, for determining the structure of an unknown compound.

You can also use a Tee union to direct samples from the syringe pump into an LC flow (without a column), which then enters the MS detector. This technique is used to introduce sample at a steady rate at higher solvent flow rates for tuning in ESI or APCI on an analyte of interest. You can also use this technique to introduce a solution of pure analyte at a steady rate in ESI or APCI.

You can introduce samples from a syringe into the loop of the injector valve. You can then use the valve to introduce the sample into an LC flow, which then enters the MS detector. This technique is used in ESI or APCI to introduce pure analytes into the MS detector in a slug. It is useful when you have a limited quantity of pure analyte.

You can also use an LC autosampler to introduce samples into an LC flow. This technique is also used in ESI or APCI to introduce a solution of pure analyte into the MS detector in a slug.

Finally, you can use an LC autosampler to introduce a mixture onto an LC column. This technique is used with ESI or APCI to separate the analytes before they are introduced sequentially into the MS detector.

You can refer to subsequent chapters in this manual and to **LCQ Advantage Getting Connected** for plumbing diagrams for methods of sample introduction.

Table 1-1. Sample introduction techniques

	Sample Introduction Technique	Analytical Technique	Figure Reference
Syringe Pump Flow (no LC Flow)	Syringe pump*	ESI automatic tuning and calibrating ESI analysis of a pure analyte solution	**LCQ Advantage Getting Started** Figure 2-5
LC Flow Without Chromatographic Separation (no column)	Syringe pump into LC flow (connected by Tee union)**	ESI or APCI automatic optimization of tuning on analyte of interest ESI or APCI analysis of a pure analyte solution	**LCQ Advantage Getting Started** Figure 4-1 (ESI) Figure 6-1 (APCI)
	Loop injection into LC flow	ESI or APCI analysis of a pure analyte solution	**LCQ Advantage Getting Started** Figure 5-1 (ESI) Figure 8-1 (APCI)
	Autosampler injection into LC flow (one or multiple injections)	ESI or APCI analysis of a pure analyte solution	**LCQ Advantage Getting Connected** Figure 11-6 (ESI) Figure 11-9 (APCI)
LC Flow With Chromatographic Separation	Autosampler injections into LC column via LC flow (one or multiple injections)	ESI or APCI analysis of mixtures	

Provides steady state introduction of sample (direct infusion)

1.5 What Types of Buffers Should I Use? What Types Should I Avoid?

Many LC applications use nonvolatile buffers such as phosphate and borate buffers. It is best to avoid use of these nonvolatile buffers with the MS detector, however, causes the following problems:

- Blocking the capillary in the probe
- Causing salt buildup on the spray head and thus compromises the integrity of the spray

Use volatile buffers when you use the MS detector. Many volatile buffer solutions are available that can be used instead of nonvolatile ones. Volatile buffer solutions include the following:

Trifluoroacetic acid

Acetic acid

Ammonium acetate

Ammonium formate

Ammonium hydroxide

- Triethylamine (TEA)

Whenever possible, use volatile buffers when you use the MS detector.

1.6 How Should I Set Up the MS Detector for Various LC Flow Rates?

The ESI probe can generate ions from liquid flows[1] of 1 µL/min to 1.0 mL/min. This flow rate range allows you to use a wide range of separation techniques, such as, CE, CEC, capillary LC, microbore LC, and analytical LC.

The APCI probe can generate ions from liquid flows[2] of 50 µL/min to 2.0 mL/min. This flow range allows you to use microbore LC, analytical LC, and semipreparative LC.

As you change the rate of flow of solvents entering the MS detector, you need to adjust several of the MS detector parameters. Specifically, for ESI you need to adjust the temperature of the ion transfer capillary and adjust the gas flow rates for the sheath gas and AUX/Sweep gas. And for APCI, you need to adjust the ion transfer capillary temperature and vaporizer temperature and adjust the gas flow rates for the sheath gas and AUX/Sweep gas.

In general, an increase in the rate of liquid flowing into the MS detector, requires a higher temperature of the ion transfer capillary (and vaporizer) and higher gas flow rate.

> **Note.** The syringe pump on the LCQ Advantage operates at a single speed. The the volume of your syringe determines the flow rate of solution into the MS detector. Specifically, the syringe pump delivers solution into the MS detector at a rate equal to 1% of the total volume of a syringe per minute. For example, if you use a 500-µL syringe, solution flows into the ion source at 5 µL/min.

Table provides guidelines for ESI operation for ion transfer capillary temperatures and gas flow rates for various LC solvent flow rates.

Table 1-3 provides guidelines for APCI operation for the ion transfer capillary temperature, vaporizer temperature, and gas flow rate for a range of LC solvent flow rates.

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[1] The ESI probe can generate ions from liquid flows of as low as 1 µL/min. However, flows below 5 µL/min require more care, especially with the position of the fused silica sample tube within the ESI probe.

[2] For the APCI probe, flows below 200 µL/min require more care to maintain a stable spray.

Table 1-2. Guidelines for setting operating parameters for LC/ESI/MS

LC Flow Rates	Suggested Column Size	Ion Transfer Capillary Temperature	Sheath Gas	Auxiliary or Sweep Gas*
Infusion or LC at flow rates of < 10 µL/min	Capillary	Typical setting: 150 to 200 °C	Not required Typical setting: 5 to 15 units	Not required Typical setting: 0 units
LC at flow rates from 50 to 200 µL/min	1 mm ID	Typical setting: 200 to 275 °C	Required Typical setting: 20 to 40 units	Not required, but might help depending on conditions Typical setting: 0 to 20 units
LC at flow rates from 100 to 500 µL/min	2 to 3 mm ID	Typical setting: 250 to 350 °C	Required Typical setting: 40 to 60 units	Not required, but usually helps to reduce solvent background ions Typical setting: 0 to 20 units
LC at flow rates from 0.4 to 1 mL/min	4.6 mm ID	Typical setting: 300 to 400 °C	Required Typical setting: 60 to 100 units	Required Typical setting: 10 to 40 units

* Note: Be sure to choose either Auxiliary gas or Sweep gas according to the hints in the topic **Should I Use Auxiliary Gas or Sweep Gas?** Auxiliary and Sweep gas cannot be used simultaneously.

Table 1-3. Guidelines for setting operating parameters for LC/APCI/MS

LC Flow Rate	Ion Transfer Capillary Temperature	Vaporizer Temperature	Sheath Gas	Auxiliary or Sweep Gas
LC at flow rates from 0.2 to 2 mL/min	Typical setting: 150 to 225 °C	Typical setting: 400 to 550 °C	Required Typical setting: 40 to 100 units	Not required, but usually helps to reduce solvent background ions Typical setting: 0 to 20 units

1.7 What Is Tuning and Calibration of the MS Detector All About?

You tune and then calibrate the MS detector in the ESI mode with calibration solution to ensure its optimum performance.

You can then optimize the tune of the MS detector with your analyte of interest in either the ESI or APCI mode, if you want to maximize the detection of one or more particular ions. (It is sometimes possible to acquire qualitative data without optimizing the parameters, but detection sensitivity is compromised.)

> **Note.** Before you calibrate, you need to tune to demonstrate that the transmission of ions into the MS detector is optimum. To perform this tune, you use the automatic tuning procedure in the Tune Plus window while infusing a calibration solution into the MS detector at a *steady rate* of 5 µL/min for several minutes. You choose one of the ions in the calibration solution that is closest to the mass-to-charge ratio of interest in your analyte. You observe the Tune Plus window as Xcalibur tunes your LCQ Advantage automatically. You then go on to perform a calibration.

Calibration parameters are instrument parameters whose values do not vary with the type of experiment. It is recommended that you calibrate the MS detector about once every three months and that you check the calibration about once a week. (To perform a calibration, in almost all cases, the settings in the existing Calibration file are a sufficient starting place.)

Automatic and semi-automatic calibration (including checking the calibration) require that you introduce calibration solution into the MS detector at a *steady rate* while the procedure is running. You introduce the solution directly from the syringe pump into the MS detector in the ESI/MS mode.

Tune parameters are instrument parameters whose values can vary with the type of experiment. For example, if your experiment requires quantitative data on one or more particular ions, you need to tune the MS detector with your analyte if you change any one of the parameters specific to the experiment or analyte.

Note. The most important parameters that affect the signal quality during ESI/MS operation are the ion transfer capillary temperature, capillary voltage, tube lens offset voltage, gases, and solution flow rate. If any one of these parameters is changed, you need to reoptimize MS detector parameters.

Automatic and semi-automatic tuning procedures (including optimizing the collision energy) require that you introduce calibration solution, or a tuning solution of your analyte of interest, into the MS detector at a steady rate in either of two ways:

- Introduce the solution directly from the syringe pump. Refer to the topic: **Setting Up the Syringe Pump for Tuning and Calibrating.**
- Introduce the sample from the syringe pump into the effluent of the LC by using a Tee union. Refer to the topic: **Setting Up to Introduce Sample by Syringe Pump into Solvent Flow from an LC.**

The first method is good for tuning if you intend to use an experiment type at a low flow rate involving the syringe pump. The second method is useful if you intend to use an experiment type at a higher flow rate involving the LC. However, the second method of introduction puts a comparatively large amount of analyte into the MS detector. Therefore, before you can perform an analytical run to analyze for the analyte, you might need to clean the API spray shield.

Caution. Do not use calibration solution at flow rates above 10 µL/min. Ultramark 1621 can contaminate your system at high concentrations.

In most cases, you can use the tune you obtain from the automatic or semi-automatic tuning procedures for your analytical experiments. However, for some applications, you might need to tune several MS detector parameters. In that case, you would tune manually. With the manual tuning process, you introduce a tuning solution at a steady rate.

It is best to tune with the MS detector in the same operational mode as that for the analytical experiment.

Table 1-4 summarizes methods of sample introduction for each of the calibration and tuning procedures.

Table 1-4. Summary of methods of sample introduction for calibration and tuning

	Calibrating			Tuning			
Sample/ Sample Intro	**Check**	**Auto**	**Semi-auto**	**Auto**	**Semi-auto**	**Manual**	**Collision Energy**
Calibration solution/ Syringe pump	✓	✓	✓	✓	✓	✓	✓
Your tune solution/ Syringe pump				✓	✓	✓	✓
Your tune solution/ Syringe pump into LC flow by using Tee union				✓	✓	✓	✓

1.8 What Types of Experiments Can I Perform with LCQ Advantage?

This topic describes several types of experiments that you can perform with LCQ Advantage. The experiments can be grouped into two categories:

- General MS or MS/MS
- Data-Dependent™

You can specify which type of experiment you want to perform in the Instrument Setup window, and then save it in an Instrument Method (*.meth*) file.

> **Note.** Procedures for these experiments are beyond the scope of this **LCQ Advantage Getting Started** manual. If you need more information, refer to online Help.

General MS or MS/MS Experiments

General MS or MS/MS

A General MS or MS/MS experiment is best used for the quantitative analysis of known compounds. However, you can also use a General experiment to collect qualitative data for structural analysis. Xcalibur includes an Instrument Method template in Instrument Setup so you can get started with a General MS or MS/MS experiment. See Figure 1-1 for an example of a General MS or MS/MS experiment template.

In a General *MS* quantitation experiment, you need to specify the mass range of your analyte(s) of interest. In a General *MS/MS* quantitation experiment, you need to specify a parent (precursor ion) that fragments into distinctive product ions. In a General MS/MS quantitation experiment, you need to specify the mass-to-charge ratios of *all* the parent ions of interest. LCQ Advantage can then collect data on the ions in the range or on the product ions of the parent ion(s) that you specify.

If you use a General experiment to collect data for qualitative (structural) analysis, you specify the scan mode (MS or MS/MS) for which you want data in the Scan Event Settings group box. If you specify MS/MS, you then choose the parent ion(s) for which you want data in the Set Parent List dialog box. LCQ Advantage can then collect distinct qualitative information for structural analysis or for spectral reference.

The LCQ Advantage can generate reproducible, product-specific spectra, even from laboratory to laboratory. Consequently, reference spectra that are generated with the LCQ Advantage can be used to confirm structures of compounds generated with other LCQ Advantage systems.

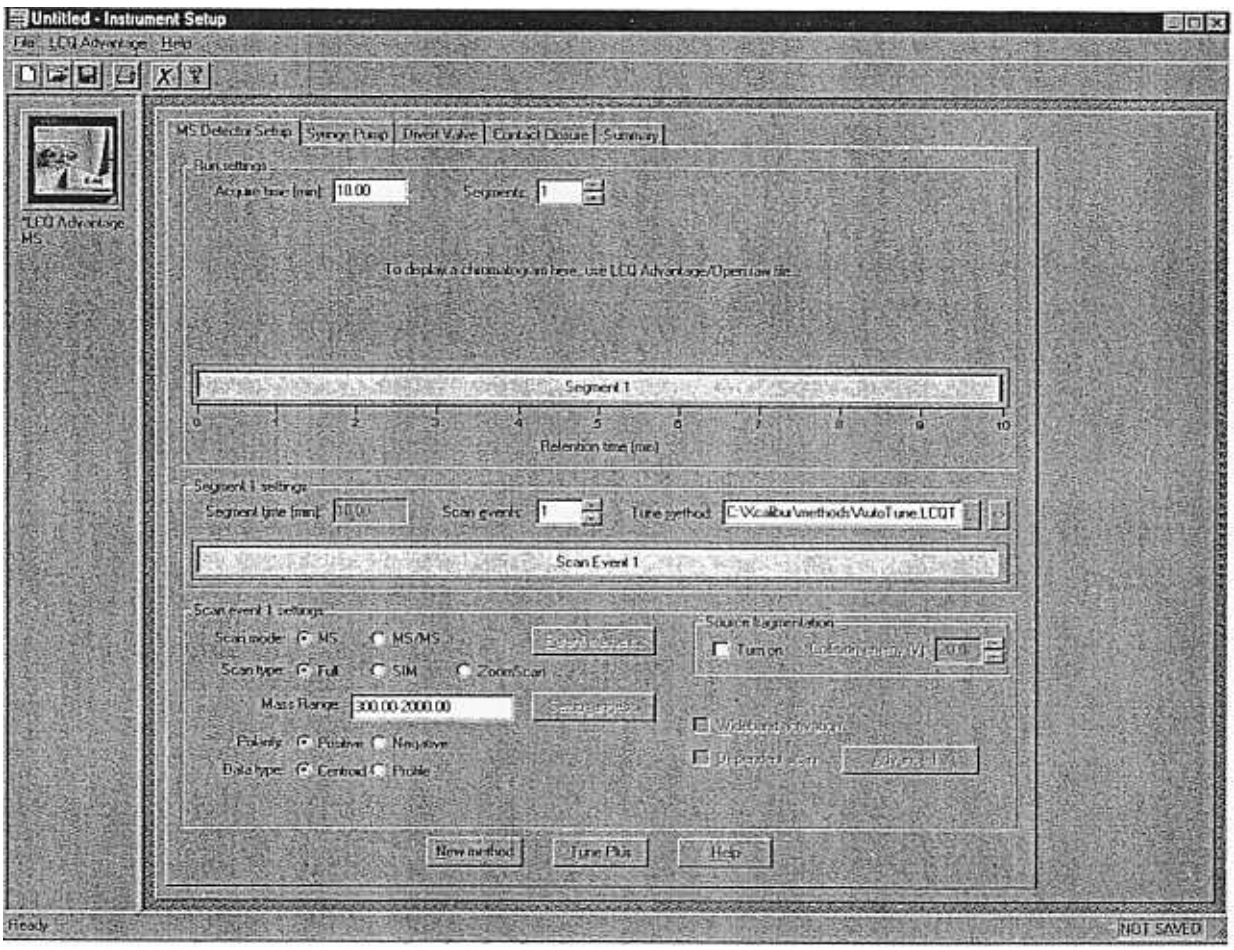
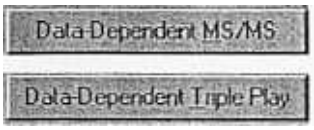


Figure 1-1. MS Detector Setup page in Instrument Setup, showing a template for a General MS or MS/MS experiment

Data-Dependent Experiments



A Data-Dependent experiment is best used for the qualitative analysis of unknown compounds for structure elucidation or confirmation. The LCQ Advantage uses the information in a Data-Dependent experiment to make decisions about the next step of the experiment automatically—without input from a user. Instrument Setup contains the Instrument Method templates that you need to get started with Data-Dependent experiments. See Figure 1-2 for an example of a Data-Dependent Triple Play experiment template.

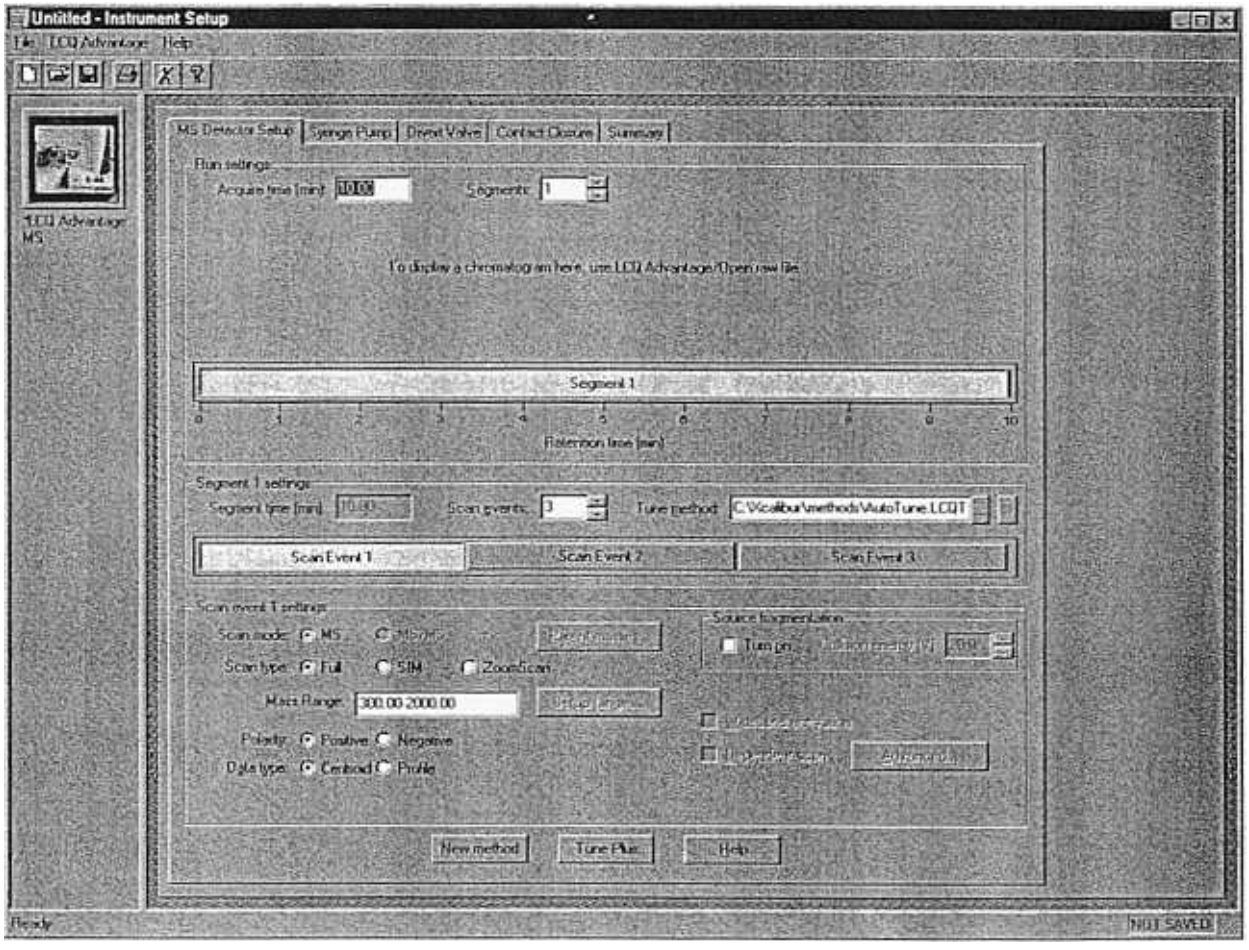


Figure 1-2. MS Detector Setup page in Instrument Setup, showing a template for a Data-Dependent Triple Play experiment. (To select a scan event that makes active the Dependent Scan checkbox, you click on either the Scan Event 2 or Scan Event 3 button.)

A Data-Dependent experiment produces a great deal of data from a single sample analysis. You can run a Data-Dependent experiment even if you know very little about your sample, and even if you are unfamiliar with the variables of mass spectroscopy. In a Data-Dependent experiment, you can specify parent ions for fragmentation or you can let LCQ Advantage automatically select the ions for fragmentation. LCQ Advantage can collect the structural information for every parent ion in the sample automatically, even if the sample is a mixture of compounds.

A Data-Dependent experiment requires minimal input from a user about how the experiment should best proceed. The user specifies that one or more scan events of an experiment segment are to be run as Data-Dependent. Then, LCQ Advantage collects MS/MS data and makes decisions about what the next step in the experiment should be to collect even more data. For example, in a Data-Dependent Triple Play experiment for a mixture of compounds, LCQ Advantage can decide which parent ion to isolate, the charge state of the parent ion, and the molecular weight of the compound.

You can approach the setup of Data-Dependent experiments in either of two ways:

If you have some idea of the parent ion, or if you expect a certain kind of parent, you can set up a list of possible parent ions. Then, when one of the parent ions you specified is detected, you can acquire product spectra and analyze the information. Conversely, you can also set up a list of ions that you do *not* want to be selected for fragmentation.

If you have little information about your compound, you can set up the parameters of a Data-Dependent experiment so that if the intensity of the ion signal is above a specified threshold, LCQ Advantage generates product spectra. Later, you decide if the information is useful. Parameters that you might specify, for example, include threshold values for the intensity of the MS or MS/MS ion signal. Whatever threshold values you choose should accomplish the isolation of your parent ions of interest.

You can find useful structural information about your compound automatically with the simplest Data-Dependent experiment, Data-Dependent MS/MS. You specify the MS scan range, and you do not even need to specify a parent ion. LCQ Advantage can then collect full scan MS data, pick the most intense parent ion in the spectrum, and fragment the ion to generate product ions.

A Data-Dependent Triple-Play experiment is the same as Data-Dependent MS/MS, but includes the identification of the charge state of the parent with the LCQ Advantage ZoomScan feature. A Data-Dependent Triple-Play experiment collects full scan MS data, and then uses ZoomScan to determine the charge state of the parent ion and calculate the molecular weight. The parent ion is then fragmented into product ions (MS/MS). For example, if LCQ Advantage determines a charge state equal to 2, and if the mass-to-charge ratio of the parent ion is *m/z* 500, then the mass-to-charge ratios of the product ions can be up to *m/z* 1000 (or 2 × 500).

You can use a Data-Dependent experiment (from templates in Instrument Setup) to do the following:

- Identify low-level impurities in high-purity compounds (Data-Dependent MS/MS)
- Identify metabolites in a complex mixture (Chromatographic Separation with Data-Dependent MS/MS)

You can use a Data-Dependent MS/MS experiment to identify process impurities. In the quality assurance process for aspirin, for example, the LCQ Advantage can identify impurities of 0.1%.

A Data-Dependent MS/MS experiment of a complex mixture of drug metabolites can provide highly specific structural information. Characteristic masses along the metabolic pathways of a drug, for example, can produce MS/MS spectra that are specific to the structure of the drug. These spectra are essential in metabolite identification.