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# The benefits of adding single quadrupole mass detection to liquid chromatography

Authors: Dan Kalu Appulage[1] and Stephan Meding[2]; [1]Thermo Fisher Scientific, Austin, TX, USA; [2]Thermo Fisher Scientific, Germering, Germany

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## Goal

Provide an overview of single quadrupole mass detection for liquid chromatography and its value to chromatographers

## Introduction

Optical detection, such as ultraviolet (UV) or fluorescence detection (FLD), has long been the standard detection type for most chromatographers due to its method robustness, ease of use, and low maintenance. However, UV detection comes with several drawbacks: the inability to detect analytes that lack a chromophore, the challenge to quantify partially resolved peaks, and a lack of sufficient selectivity for the identification of unknowns. The Thermo Scientific™ ISQ™ EC and ISQ EM single quadrupole mass detectors directly solve these challenges, while hardware and software tools allow chromatographers to use them with the same simplicity as UV detectors.

## How a mass detector works

Mass detectors consist of three main components (Figure 1). The ion source produces charged analyte molecules and removes the mobile phase through evaporation and desolvation to result in free ions in the gas phase. The mass analyzer consists of two parts: the ion guide system and the quadrupole. The ion guide system filters off neutrals and carries the ions through the vacuum chamber to the quadrupole. Collision induced dissociation takes place in this region. The quadrupole focuses and separates the ions according to their mass to charge ratio *(m/z)*. The ions are then detected by the ion detector, which usually consists of a dynode and an electron multiplier to amplify the ion signal. The signal is then processed and displayed as a mass spectrum where the intensity of the ions is shown either as ion counts or relative abundance against the mass-to-charge *(m/z)* ratio.

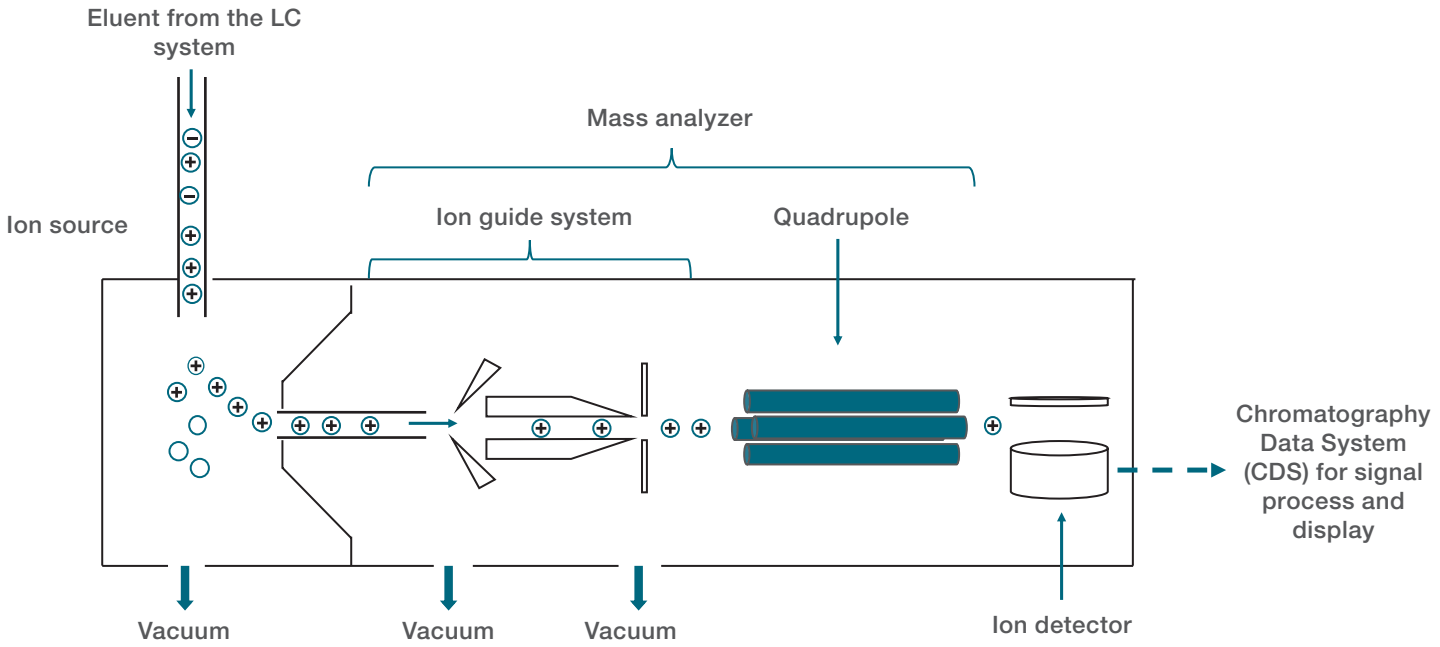



**Figure 1. Schematic diagram of a mass detector.** It comprises the ion source, the mass analyzer that can be split into the ion guide and the quadrupole, and the ion detector.

Ion sources can work under different conditions. The most commonly used mode is called atmospheric pressure ionization (API) where the ion source is kept under atmospheric pressure. The two most common API modes are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), both of which will be discussed in more detail later.

There are different types of mass analyzers, such as quadrupole, time of flight, ion trap, and Orbitrap™ mass analyzers. Tandem mass analyzers combine two or more mass analyzers and are used for more complex analyses. Here we focus on single quadrupole mass analyzers, like the ISQ EC and ISQ EM, since they are common detectors for routine liquid chromatography mass spectrometry (LC-MS) analyses. While the *m/z* separation mode differs between mass detectors, the actual ion detection system is typically the same. A dynode and an electron multiplier are combined to create a current that is proportional to the number of ions for a particular *m/z* ratio hitting the detector.

## Key advantages of mass detection

There are various reasons why a chromatographer who has been using an optical detector such as a UV detector should consider adding a mass detector to their LC system:

- A mass detector provides the *m/z* ratio of an analyte, which allows inference of the analyte mass. This helps users to identify the analyte with more confidence than solely relying on the retention time and UV spectrum. In addition, structural information can be gained through the molecular mass, the isotopic distribution, and by fragmentation of the molecule and the molecular mass of the fragments.
- A mass detector selectively detects co-eluting compounds. Peak purity can then be easily determined, and quantitation can be done on each analyte separately. Since these co-eluting analytes can be distinguished, the need for additional sample preparation is no longer mandatory.
- A mass detector is typically more sensitive than a UV detector, reducing the need for enrichment steps or large volume injections.
- A mass detector can detect analytes that lack a chromophore or only have a poor chromophore. The only limitations are that the analytes need to be ionizable and solvents must be volatile.

## What should be considered when an LC is coupled with a mass detector?

When a mass detector is added to the LC system there are certain things that a chromatographer needs to think about. In chromatography there are different solvents, additives, and modifiers used in the mobile phases to obtain the required resolution of the analytes of interest. One of the major things to keep in mind for mass detection is using compatible mobile phases and additives. Since the mass detector requires the eluent to be in the gas phase, all components of the eluent must be volatile. For example, acetic acid, formic acid, ammonium formate, or triethylamine can be used as additives since they are volatile. Nonvolatile additives, such as phosphates, should be avoided to prevent source fouling.

**Table 1. A list of commonly used LC solvents and additives that are compatible with MS detection**

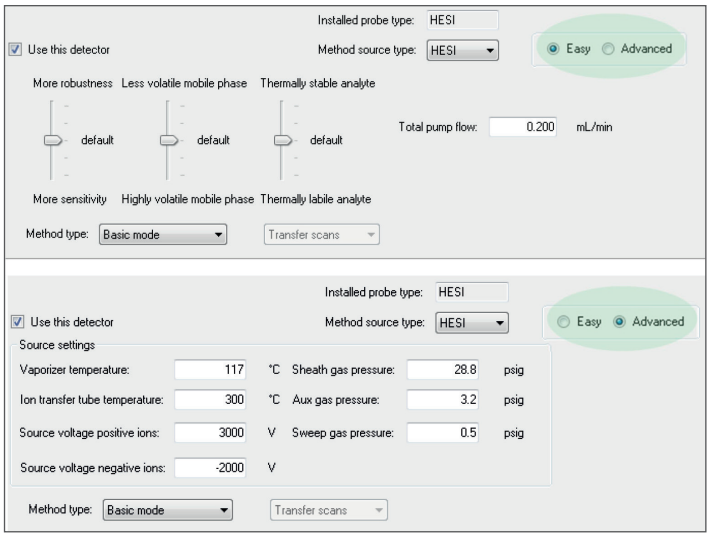
| Solvents | Additives |
|---|---|
| Water | Acetic acid |
| Acetonitrile | Formic acid |
| Methanol | Ammonium hydroxide |
| Ethanol | Ammonium formate* |
| Propanol | Ammonium acetate* |
| Isopropanol | Triethylamine |

*It is recommended to keep the salt concentration relatively low (e.g. ≤10 mM)

## How is data generated using a mass detector?

Creating an MS method with an ISQ EC or ISQ EM mass detector is just as simple as setting up an LC-UV method. Like other LC detectors, the ISQ EC and ISQ EM are both fully integrated into Thermo Scientific™ Chromeleon™ (CDS) Chromatography Data System. With the introduction of Thermo Scientific™ AutoSpray smart method setup, where the mass detector parameters are automatically set by the software depending on the LC flow rate, and the analyte and eluent properties, even a user without previous MS experience can achieve a successful MS analysis from the very first time. Further, there are two different modes called Easy and Advanced Mode that users can choose depending on the type of the analysis. In the Easy Mode, sliders are available for adjusting the mass detector parameters, such as source gas flow and the source temperature settings to matrix, sample, and eluent properties, and there is an advanced option for expert users who want to further optimize the source settings (Figure 2).

A HESI




B APCI

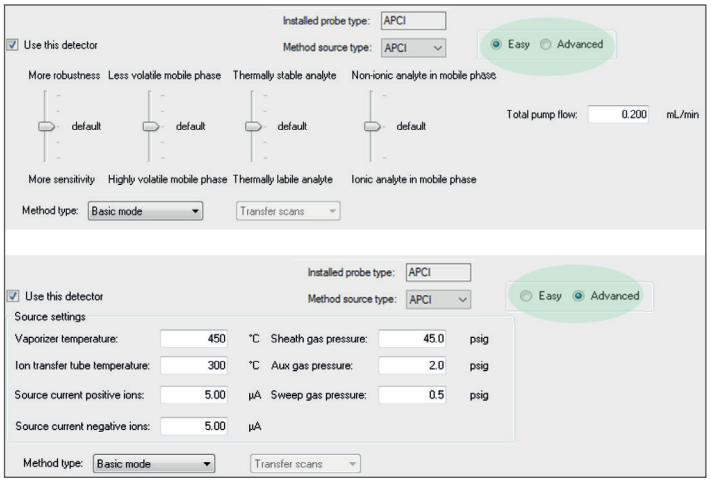



**Figure 2. Automated MS method setting for heated electrospray ionization (HESI) mode (A) and APCI mode (B).** With AutoSpray technology, Easy Mode enables even users without prior MS experience to easily set up their methods (top). Advanced Mode gives full access to the source parameters, enabling experienced users to further refine their methods (bottom).

## Tuning and maintenance of a mass detector

The ISQ EC and ISQ EM mass detectors are equipped with an integrated calibrant delivery system and an autotune program that can be run with a few clicks from the ePanel or within a sequence queue. Both mass detectors require very little maintenance. The most common maintenance procedure is removing, cleaning, and replacing the ion transfer tube and the sweep cone. It can be performed in less than an hour without breaking the vacuum and therefore, instrument downtime is minimal. Further, there is a fully automated diagnostic-only tuning program available that helps users to troubleshoot the system.[1]

## Atmospheric pressure ionization modes

The two commonly used API modes, ESI and APCI, are regarded as "soft" ionization processes because they produce ions while transferring relatively little energy, and as a result, little or no molecule fragmentation occurs during ionization. ESI mode is a very soft ionization method where even large proteins can be ionized. APCI is a bit harsher and, therefore, it is suitable for small molecules only.

In ESI mode (Figure 3), the analyte molecules are ionized as they leave the solution. They acquire charges from the evaporating charged droplets generated from the HESI needle. So, additives such as acids or bases in the mobile phase provide the required charges and thus, help analyte ionization. The analytes are then present as protonated cations $[M+H]^+$ or deprotonated anions $[M-H]^-$. The eluent is introduced to the electrospray interface using a capillary and a potential is applied between the capillary and the sampling cone. The formation of the gas phase ions and evaporation of mobile phase is complex and there are many review articles that discuss the process in great detail such as Konermann *et al*.[2]

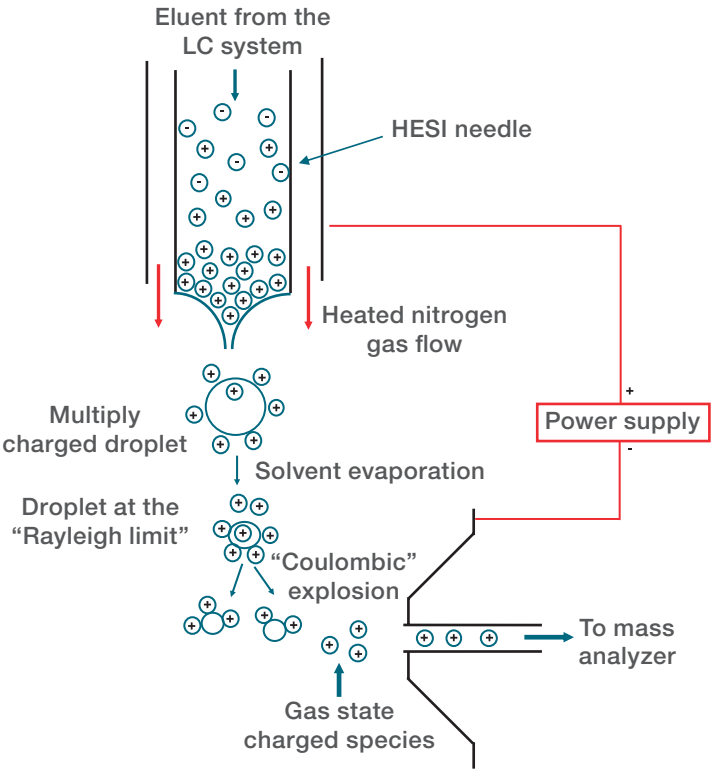



Figure 3. Schematic diagram of ESI process

In the case of APCI mode (Figure 4), the eluent is introduced to the interface using a capillary but there is no electrical potential applied to the capillary. The liquid emerges from the capillary and is sprayed into a heated region while a heated nebulizing gas flow surrounds it. A pin is placed between the heated region and the entry to the mass analyzer and a high potential is applied to it to produce a discharge. Analyte molecules evaporate from the liquid droplets and are ionized in the gas phase through interactions with ions created from the discharge process. A full description of the ionization process is beyond the scope of this technical note and the details of the ionization process can be found in the literature.[3,4]

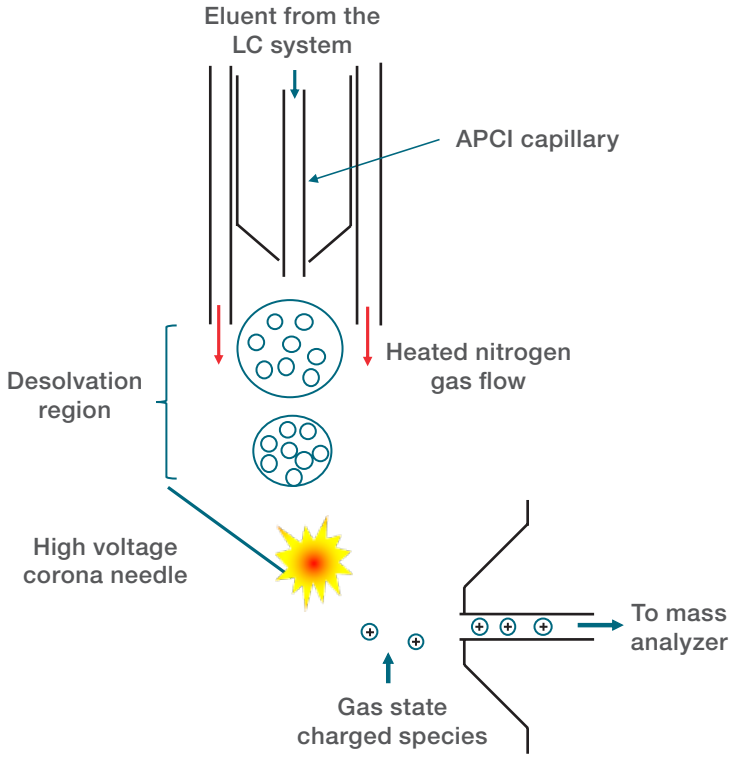



Figure 4. Schematic diagram of APCI process

With ESI mode, some analyte molecules carry multiple charges since the charge state depends on how many protons a molecule can acquire or shed (e.g., peptides, proteins, oligonucleotides), whereas with APCI mode, only singly charged analyte ions are formed. Table 2 summarizes the favorable ionization mode for different compound classes, and Figure 5 shows that ESI favors mid polar to polar analytes with a wider range of molecular weights, whereas APCI favors low to mid polar and relatively low molecular weight analytes.

Table 2. Suitable API mode for different compound classes for mass detection

| Compound class | ESI | APCI |
|---|---|---|
| DNA | ** | - |
| Proteins | *** | - |
| Peptides | *** | - |
| Amino acids | ** | * |
| Polymers | ** | * |
| Carbohydrates | ** | ** |
| Triglycerides | * | *** |

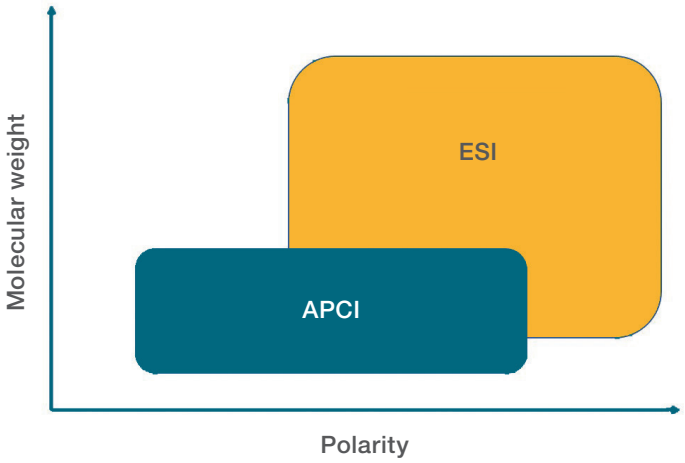



Figure 5. Most commonly used ionization modes with the applicability based on the analyte polarity and molecular weight

## Quadrupole mass analyzer

Quadrupoles are the most common mass detectors due in part to their simplicity, relatively low costs, and robustness. Quadrupole mass analyzers, as the name suggests, have four metal rods that are placed in parallel. Direct current (DC) and radio frequency (RF) potentials are applied to opposite rods. To detect an ion with a specific *m/z*, the DC and RF voltages are set to predefined values. This results in a stable vibratory motion of ions with that distinct *m/z*. They pass through the quadrupole, while all other ions have an unstable trajectory and exit through the sides of the quadrupole and are thereby removed (Figure 5). This mode is called selected ion monitoring (SIM) and is similar to measuring absorbance at a specific wavelength using a UV detector. For acquiring a mass spectrum, the DC and RF voltages are ramped through so that different *m/z* values are scanned consecutively, resulting in a single scan event. However, the different *m/z* are scanned consecutively not in parallel. As a rule of thumb, the scan rate should be chosen so that there are enough data points across a chromatographic peak. Choosing a faster scan speed results in a lower scan time per *m/z* window and this may lower the detection sensitivity.

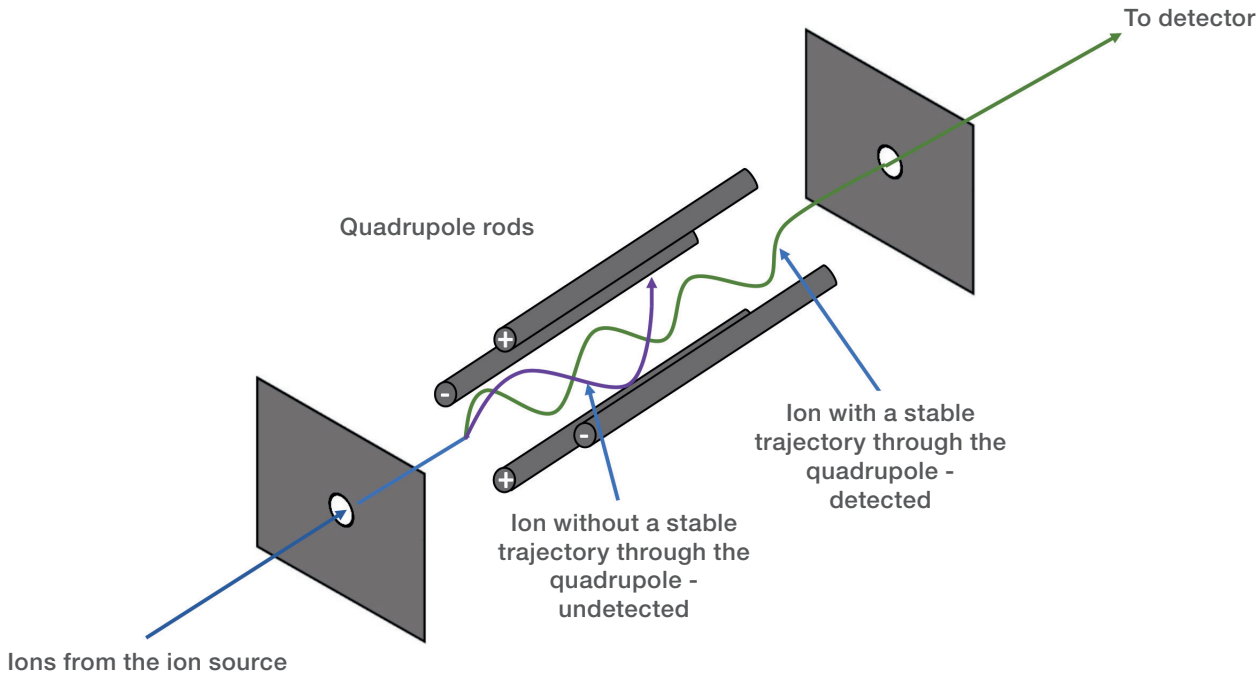



Figure 6. Schematic diagram showing how a quadrupole mass analyzer works

## Chromatographic resolution vs. mass resolution

When discussing resolution, there are two different resolutions that need to be discussed in LC-MS. Chromatographic resolution indicates how two component bands are separated from each other. Mass resolution on the other hand is different from chromatographic resolution. It determines whether the detector can resolve two *m/z* signals from one another. Most common quadrupole mass analyzers give 1-unit mass resolution, meaning that it can separate two *m/z* signals that differ by 1 Da at the base or 0.7 Da at full width half maximum of the peak. Please note that a charge state of z = 1 is assumed.

## Common adducts in mass detection

Adducts are molecules or ions present in the sample or mobile phase that bind non-covalently to the analytes. Salt ions, such as $Na^+$, $K^+$, $NH_4^+$, $CH_3CO_2^-$, and $Cl^-$, are common ionic adducts. For cations, they usually replace the proton being added. For anions, they bind to the neutral molecule. The mobile phase or solvent matrix itself can also form adducts. They have no charge themselves and result in an additional molecular weight of one or multiple times their weight depending on how many of them bind to the analyte molecule. These adducts can be observed in ESI and APCI ionization mode and for positively or negatively charged molecules. However, they are more prevalent when operating in the ESI ionization mode. Adduct formation can reduce the sensitivity of mass detection because an analyte is present in multiple forms, reducing the intensity of each of them, and can increase the complexity of the mass spectrum. With the source collision induced dissociation capabilities available in the ISQ EC and ISQ EM mass detectors, the presence of adducts can often be reduced by removing them from the analyte while keeping the analyte intact.

## What are isotopes and their effect on mass detection?

Isotopes of a given element consist of the same number of protons but a different number of neutrons. Therefore, isotopes have different masses while having the same chemical properties, so they elute at the same retention time. Since in mass spectrometry the *m/z* is measured, different isotopes of the same ion are observed as different peaks, if the resolution of the mass spectrometer is sufficient to resolve them. The intensity is proportional to the natural abundance of the corresponding isotope (unless isotopically labeled analytes are measured). It is common practice in chemistry to calculate the mass of a molecule using the average atomic masses. Mass spectrometry is an analytical technique selective at the molecular weight level. For example, analysis of bromine would give two equally intense peaks at *m/z* 79 and 81. So, analytes containing a single bromine will also show this intensity pattern of two equally intense *m/z* species 2 Da apart. This can be used for further analyte confirmation. Other elements also have distinct isotope patterns which can be used for analyte identity confirmation (Table 3).

**Table 3. Isotopes commonly encountered in mass detector analysis with their masses and natural abundances[3]**

| Isotope | | Mass (Da) | Natural abundance |
|---|---|---|---|
| **Hydrogen** | $^{1}H$ | 1.007825 | 99.985 |
| **(Deuterium)** | $^{2}H$ | 2.014102 | 0.015 |
| **Carbon** | $^{12}C$ | 12.00000 | 98.90 |
| | $^{13}C$ | 13.003355 | 1.10 |
| **Nitrogen** | $^{14}N$ | 14.003074 | 99.63 |
| | $^{15}N$ | 15.000109 | 0.37 |
| **Oxygen** | $^{16}O$ | 15.994915 | 99.76 |
| | $^{17}O$ | 16.999131 | 0.04 |
| | $^{18}O$ | 17.999160 | 0.20 |
| **Phosphorus** | $^{31}P$ | 30.973770 | 100.00 |
| **Sulfur** | $^{32}S$ | 31.972072 | 95.02 |
| | $^{33}S$ | 32.971459 | 0.75 |
| | $^{34}S$ | 33.967868 | 4.21 |
| **Chlorine** | $^{35}Cl$ | 34.968853 | 75.77 |
| | $^{37}Cl$ | 36.965903 | 24.23 |
| **Bromine** | $^{79}Br$ | 78.918336 | 50.50 |
| | $^{81}Br$ | 80.916289 | 49.50 |

## Ion suppression and how it affects mass detection

The impact of compounds present in either the sample matrix or the mobile phases on the ionization efficiency of the analytes of interest is called the matrix effect or ion suppression. It can be substantial due to several reasons. If ion pairing agents, acidic or basic buffers are present in the mobile phases or if contaminant species co-elute, then ion suppression can reduce the signal intensity of the analytes. This effect gets more visible when the concentration of the analyte is low.

This effect occurs at the early stage of the mass detector ionization process in the ion source. It is relatively easy to find out whether there is an ion suppression effect for a particular analyte by performing an analysis of a blank sample and a matrix sample each spiked with a standard solution and comparing the signal intensities. The difference is caused by ion suppression. Ion suppression only causes analytical issues if it is inconsistent from run to run or reduces the analytes signal below the limit of quantitation. It is known that APCI is less susceptible to ion suppression effects compared to ESI. The reason for this behavior is that ESI needs analytes to have charges available in solution. There, the total number of charges available is limited, e.g., by the proton concentration, and competition for the charges occurs when too few are available. In APCI, the charges are applied to the molecules in the gas phase through interactions with ions created by a discharge needle. There are various things that can be done to minimize the effects of ion suppression, such as selecting MS compatible and pure solvents and mobile phases, improving the sample clean up, selective analyte extraction, and optimization of chromatographic conditions to separate the elution of the analytes from suppressing compounds.

Ion suppression can also be caused by the analyte itself if the concentration is too high, and not enough charges are available for efficient ionization when the analyte elutes. The peak exhibits a flattened apex or can even be split instead of having an apex. In this case, reducing the amount of analyte usually resolves the issue. Alternatively, a higher concentration of additive can be tested. If none of the approaches are working, there are other methods, such as the standard addition method or having an internal standard to overcome the ion suppression effect on the quantitative analysis.[5]

If only SIM is used in a particular method, the presence of ion suppression effects can go unnoticed. Therefore, full scans or a combination of SIM and full scans should be performed during the early stages of method development.

## Specifications and applications of ISQ EC and ISQ EM single quadrupole mass detectors

The ISQ EC and ISQ EM instruments are versatile single quadrupole mass detectors. They have mass ranges of 10–1,250 *m/z* and 10–2,000 *m/z*, respectively, and can be used for flow rates from 50 µL/min to 2.0 mL/min without splitting the flow or requiring a make-up flow. Since they can operate with 100% organic and 100% aqueous mobile phases at all specified flow rates, they can be coupled not only with LC systems but also with ion chromatography (IC) systems. Heated electrospray ionization is the only ionization (HESI) mode available for the ISQ EC mass detector. Both HESI and APCI are available for the ISQ EM mass detector. The ISQ EC mass detector is designed for reliable routine analysis of low molecular weight ions using both LC and IC for applications in environmental analysis and academia. The ISQ EM mass detector on the other hand is developed for detection and quantitation of small and large molecules with an extended mass range, and for measurement of both polar and non-polar analytes with HESI and dual HESI/APCI source options. This is well suited for applications in pharma, academia, and fine chemical markets.

The ISQ EC and ISQ EM mass detectors are fully integrated into Chromeleon CDS. Therefore, data analysis of mass spectrometric data is done in the same software as UV-based detection allowing both data sets to be analyzed and reported together. Dedicated templates for MS data analysis are available that are designed in the same way as the standard chromatography templates, simplifying the addition of mass detection to the analytical workflow.

Collaterals, specifications, brochures, and manuals can be found on the ISQ EC and ISQ EM product page (www.thermofisher.com/singlequadMS).

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## Conclusion

Mass detection helps to overcome the challenges chromatographers face in many ways.

- The selective, molecular mass-based detection helps identify the analytes and allows separation of co-eluting compounds.
- The higher sensitivity compared to UV detection results in less analyte being needed for successful analyses.
- The detection mechanisms of ionization and subsequent ion detection makes detection of analytes without or with only poor chromophores possible.

Once a chromatographer has understood which solvents and additives are suitable for mass spectrometry and is familiarized with the concepts of mass selective detection, the additional information gained will prove very useful while the amount of extra work is minimal. Mass detection provides a higher level of confidence in your analytical results.

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