IC–MS: Ion Chromatography–Mass Spectrometry

Author: Alexander N. Semyonov, Thermo Fisher Scientific, Austin, TX 78728

Keywords: ion chromatography, mass spectrometry, separation sciences, IC–MS overview, identification, quantitation, IC–MS/MS, disinfection byproducts, polar pesticides, metabolomics, metabolites, sugar phosphates

IC–MS is a modern analytical technique performed with an ion chromatograph and a mass spectrometer, IC and MS, which are connected (hyphenated) together in series (Figure 1). Each of the two instruments is fully functional and can operate independently. In that case, we can say that ion chromatography is a stand-alone (rather than hyphenated) analytical technique. Likewise, a mass spectrometer used on its own operates in «direct injection» or «direct infusion» mode, to reflect the absence of the so-called «front end», that is, the chromatography system. When the two instruments are connected and used as a hyphenated technique, there is a synergistic effect achieved. The IC–MS data from the sample are much more insightful, actionable, reliable, and accurate in comparison with each technique used independently.

Ion chromatography is a type of liquid chromatography, where the species being separated are ions, or can exist as ions as a result of dynamic equilibrium in solution. Similar to conventional High Performance Liquid Chromatography (HPLC), the separation of chemical compounds in ion chromatography takes place on the column and results in formation of dynamic (that is, moving in space and time) concentration bands eluting through and off that column. Unlike a conventional liquid chromatograph, an ion chromatograph is designed completely metal-free in the flow path with extremely low (on the order of single-digit micro Siemens) residual ionic chemical background noise. Table 1 summarizes the most important similarities and differences between conventional HPLC and IC.

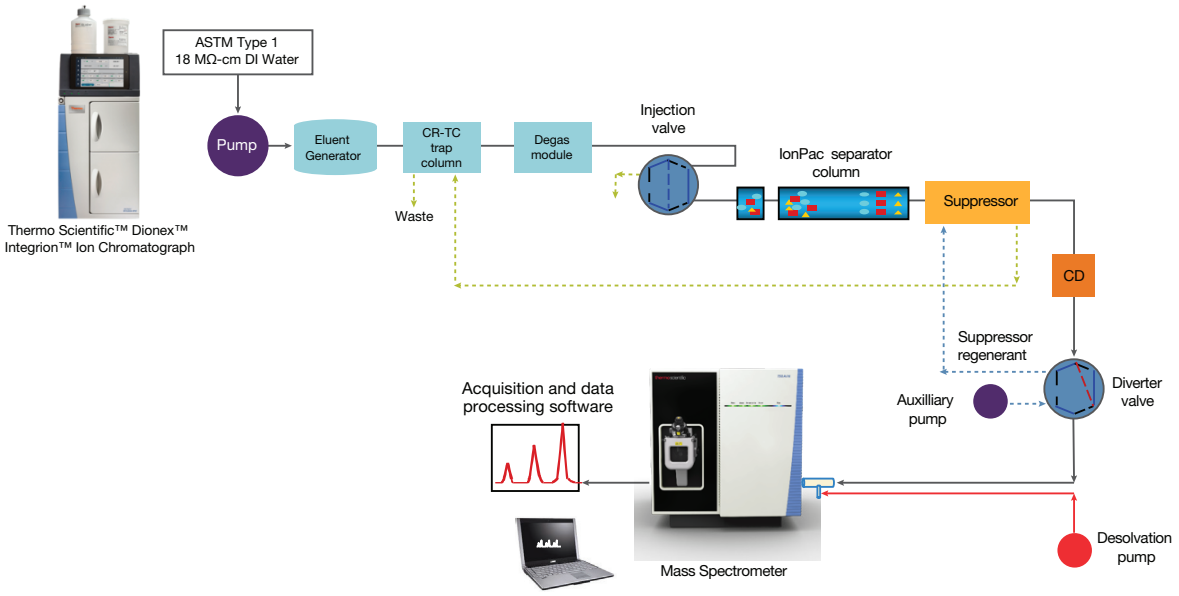


Figure 1. IC-MS schematic.

Table 1. IC vs. conventional HPLC.

Parameter	LC	IC
Stationary phase	Surface-modified (C_{18}, C_8) silica gel, mostly universal	Polymeric ion-exchange resin, application-specific
Mobile phase	Organic solvent(s) with or without water, buffers, and modifiers	Aqueous KOH or methanesulfonic acid (MSA), rarely with organic solvent
Gradient elution	Requires binary or quaternary pumps	Reagent-free IC (RFIC) systems produce gradients with an isocratic pump
Eluent generation	Not available	RFIC generates eluents using electrical current only
Metal-free flow path	No	Yes
Modifiers (formic acid, TFA)	Often required for proper interaction with and separation on the stationary phase	Not required
Salts and buffers in the column effluent	Usually present	Not present in IC with suppressed conductivity detection
Analyte classes applicability	Most soluble organic compounds	Only ionic and ionizable inorganic and organic compounds
Chemical background noise	Usually high	Usually low

The principle and the mechanism of liquid chromatography is based on the interactions between the solute (analyte dissolved in the mobile phase) and the stationary phase (column packing) that are of intermolecular (rather than covalent) nature. There are three major intermolecular interaction forces (from the weakest to the strongest): van der Waals (momentary dipole–dipole interactions), dipole–dipole interactions, and ionic (ion–ion and ion–induced dipole) interactions.

The silica-based alkyl-chain surface-modified LC columns are mostly hydrophobic. They were designed to interact with and retain nonpolar and medium polar organic compounds. The vast majority of the LC columns used today are C_{18} columns or variants thereof, collectively called «reversed phase» LC columns to distinguish them from the non–modified (normal phase, NP) silica gel columns. Increasing the dipole moment of the modifying surface groups and incorporating hydrophilic functional groups into the surface-modifying alkyl chains creates

a class of LC columns called hydrophilic interactions liquid chromatography (HILIC). Both reversed phase and HILIC columns rely on van der Waals and dipole–dipole interactions as their retention mechanism, and typically lack the ionic groups and Coulomb intermolecular interactions.

Unlike both reversed phase HPLC (RPLC) and HILIC columns, the IC columns are (a) typically hydrophilic and (b) designed to rely on the Coulombic forces (ion–ion intermolecular interactions). As such, most IC columns are complementary/orthogonal in their selectivity to all other RPLC and HILIC columns. In other words, IC columns have vastly different selectivities (the alpha term in the van Deemter model) in comparison with liquid chromatography columns (Figure 2). This fact makes the IC columns both helpful and unique to accomplish very specific applications or determine particular analytes. In addition to classical determination of inorganic anions and cations in water and other aqueous solutions, IC and IC–MS techniques are gradually gaining acceptance for the determination of polar anionic and cationic pesticides, disinfection byproducts, as well as major metabolites such as sugar phosphates.

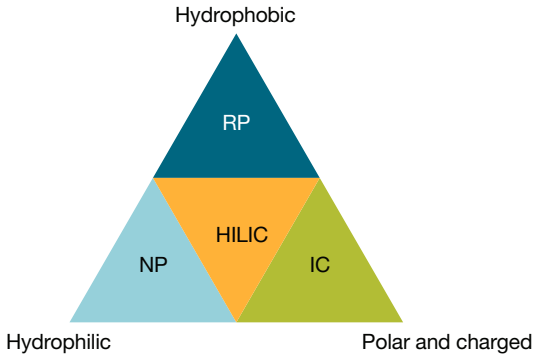


Figure 2. Triangle of LC, HILIC, and IC columns.

Modern IC is performed with suppressed conductivity detection (also known as eluent suppression), which can be implemented either as «*chemically* suppressed» or «*electrolytically* suppressed» conductivity detection. The electrolytically suppressed conductivity dominates due to simplicity, reproducibility, and reagent-free (and thus, chemical waste-free) nature. The suppressed conductivity detection feature of ion chromatography is also what makes it one of the most compatible and useful form of liquid chromatography front-end to be coupled with MS. To implement suppression (also known as «desalting» in the MS field), the effluent of the IC separation column passes through a membrane device called a *suppressor*. The suppressor requires a *regenerant flow* on the other side of the membrane: either a flow of an external chemical (for chemical suppression) or a flow of deionized water split into the regenerant ions by an electric field (for electrolytic suppression). In either case, the conductivity suppression facilitates the directional migration/transport of the counter ions other than the ions of interest and results in their selective removal. Thus, when an anion-exchange column separates anions (with metal cations present as the counter ion), the suppressor exchanges all metal cations for hydronium ions, effectively converting all salts to their corresponding acids. When a cation column's effluent passes through its suppressor, the anions are replaced by hydroxide ions, effectively converting salts into their corresponding bases. Not only does this eliminate the accumulation of salts on the mass spectrometer's interface (entrance aperture and skimmer cone), but it also prevents another phenomenon, known as ion suppression. In the ion source of the mass spectrometer additional ions from an unsuppressed IC effluent lead to a competition for charge. The result is fewer analyte ions are created and eventually detected, degrading the method detection limit. Having the same name for two separate phenomena, electrolytic suppression on the IC side and the ion suppression on the MS side, can be confusing. Just remember that electrolytic suppression in the IC helps to reduce or eliminate the ion signal suppression in the MS. This is one of the mechanisms by which the coupling of IC and MS creates synergy in IC–MS.

To realize the benefit of continuous suppression (desalting), the hardware design of IC requires an additional pump for the suppressor's regenerant flow path. This flow passes on the other side of the suppressor's semi-permeable membrane and carries away the desalted species. This additional pump can be a conventional piston type pump or simply a plumbing to the source of deionized water (or a regenerating chemical in case of chemical suppression) to push liquid through the suppressor.

For the entrenched HPLC users limited to the HPLC/HILIC columns' selectivity in the van Deemter's alpha-space, it is possible to "try out" an IC column in their existing LC stack or front end … for a run or two (i.e. one or two injections). However, the triumvirate of the IC hardware design requirements (i.e. metal-free path, eluent generation, and suppressor plumbing) necessitates properly designed, fit-for-purpose ion chromatograph hardware whenever one desires to operate IC columns on a routine basis.

IC is a nondestructive separation technique and can be, in principle and in practice, used for preparative separations, when connected to a non-destructive bulk-property detector. The mass spectrometer, on the other hand, is an inherently destructive detector and the sample that enters MS, cannot be recovered. This is due to the inherently low ionization efficiency of the MS ion sources. To partially alleviate this problem, the effluent from the IC can be split into two flow paths. In one path, the majority of the sample, if needed, can be collected and recovered for preparative purposes, while in another path a small portion of the sample flows from the IC through the splitter and into the MS for analysis. When used for a separation on a preparative scale, this is called «mass-directed fractionation» and can be easily performed on all Thermo Scientific™ IC–MS systems controlled by Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

All detectors used in stand-alone IC, are bulk property detectors. They monitor a particular physico-chemical parameter in the flow-through detection cell for the entire (although often very small) total volume of the effluent in that cell. The detector used most often, in fact almost universally, is a conductivity detector (CD). Had there been no chromatographic separation before the detector, we could not have made any conclusion on the identity of the species the conductivity of which we observe in CD. In other words, with bulk property detectors the only criteria for compound identification is the retention time. In that sense, the conductivity detector is a nonspecific bulk property detector. Selective and specific detectors, such as electrochemical (ED, PAD) and photometric (UV, DAD) also rely on a property measured from the entire detection cell's volume. This is not the case for mass-selective detectors, such as mass spectrometers.

With IC–MS, both the conductivity detector and the MS detector can be used simultaneously. The use of both detectors (CD and MS) is advantageous, for example, in water analysis, where simultaneous determination of inorganic anions both at high trace levels (up to thousands of ppm or mg/L) and low trace levels contaminants (at sub ppb or µg/L concentrations) is possible in a single injection. In food analysis, the CD signal helps to identify the retention time regions with high matrix components content and to investigate the matrix effects, in order to generate an optimal eluent ionic strength gradient to separate the matrix anions from the analytes of interest or to decide when to divert that region of the chromatogram to waste.

Mass spectrometry (MS) is an instrumental analytical technique that separates chemical compounds on the molecular level, in the ionized form, in space and time, based on their mass-to-charge (*m/z*) ratio. All modern commercial mass spectrometers contain three main architectural elements: an *ion source* where chemical compounds and matrix are ionized and partially or fully transferred into the gas phase (desolvated); a *mass analyzer*, operating invariably in high vacuum that separates the ions formed in the ion source according to their mass-to-charge ratio; and the *ion detector*.

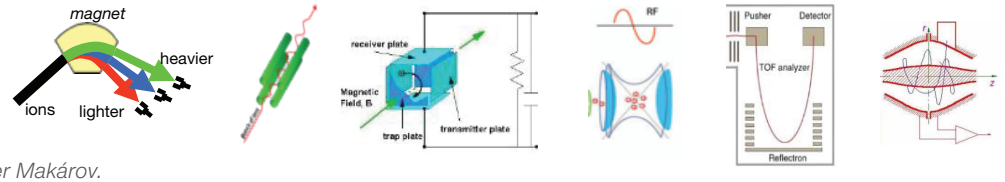
There can be numerous classifications of mass spectrometers according to the types of each of its main architectural elements and other parameters. We will consider one such classification that is the most useful for our purposes, by the mass analyzer type (Table 2). There are currently six basic mass analyzer types known: magnetic sector (e.g. Thermo Scientific™ DFS Magnetic Sector GC-HRMS), quadrupole (e.g. Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer), ion trap (e.g. Thermo Scientific™ ITQ™ Ion Trap Mass Spectrometer), time of flight (e.g. Agilent Technologies 6230), Fourier-transform ion cyclotron resonance (e.g. Bruker Daltonics 21T FT-ICR), and Thermo Scientific™ Orbitrap Mass Spectrometer, (e.g. Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer). The analyzers can be combined inside one MS instrument. In that case, the mass spectrometers are called either tandem (e.g. Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer), or hybrid (e.g. Agilent Technologies 7250 Q-TOF). It cannot be excluded that other, new types of mass analyzers may be invented in the future.

In addition to the classification by the mass analyzer type, mass spectrometers can be classified by other criteria, such as ionization sources and detectors. We will mention another type from the classification by detector type: multi-collector mass spectrometers. These detectors are designed to allow for simultaneous and therefore very precise measurement of multiple *m/z* ions at the same time, resulting in highly accurate isotopic ratios (IRMS) and ion ratio measurements. Multi-collector mass spectrometers (e.g. Thermo Scientific™ Neptune™ Series High Resolution Multicollector ICP-MS, and Thermo Scientific™ Triton™ Series Multicollector Thermal Ionization Mass Spectrometer) find their primary use in the geological, nuclear, and dating applications.

Table 2. Classification of mass spectrometers by the mass analyzer type.

Analyzer	Magnetic sector	Quadrupole filter	Fourier transform ICR	Ion trap	Time-of-flight (orth. acc)	Orbitrap
Resolution						
Transmission						
Mass accuracy						
Dynamic range						
Speed						
Quantitation						
All-ion detection						
Simplicity						
MS^n						

High
Medium
Low



Courtesy of Alexander Makárov.

When coupling an IC to MS, an electrospray (ESI), heated electrospray (HESI), or inductively coupled plasma (ICP) ion source is typically used. The resulting IC–ESI–MS hyphenated technique is used for the analysis of molecular species, while the IC–ICP–MS is used exclusively for the speciation in trace elemental analysis (TEA). The ionization efficiency of the ICP ion source is typically significantly higher than that of the ESI source, thus IC–ICP–MS is the technique of choice for element speciation applications, such as arsenic, selenium, mercury, chromium, sulfur, etc. However, all molecular species are destroyed in the plasma and thus the data from ICP–MS is limited to the elemental composition information. For the purpose of this article, the term «IC–MS» will mean IC–ESI–MS or IC–HESI–MS.

The crucial fundamental characteristic of the mass spectrometer as a mass-selective detector is that the MS is selective at the molecular level, unlike the bulk properties detectors typically used in chromatography. The selectivity at the molecular level is the basis of all other benefits of mass spectrometry (Table 3).

Table 3. Benefits of IC–MS.

Parameter	Stand-alone IC	IC–MS
Identification	by retention time	by *m/z*
Quantitation	signal-limited	chemical noise limited
Co-elution	unresolvable (except by IC×IC)	resolvable by different *m/z* values
Autointegration	difficult with shoulders and co-elutions	by SIM, SRM, XIC, and HRAM
Peak confirmation	N/A	by *m/z*
Peak identification	by retention time only	by HRAM
Confidence	bulk properties detectors based	enhanced by detecting *m/z* at the molecular-level
Limits of detection	ppm to ppb (mg/L to µg/L)	ppb to ppt (µg/L to ng/L)
ILIS internal standard	N/A	improves accuracy
MS interface desalter	N/A	IC suppressor is MS' best friend
Isobaric interferences	Cannot differentiate	HRAM
Complimentary to	HPLC	NMR
Peak capacity	Retention time domain limited	*m/z* resolution limited
Isotope-ratio (IRMS)	N/A	requires multi-collector MS

Identification with the aid of mass spectrometry is a task that can be performed in a much more reliable and confident way than relying on the retention time parameter alone, or in conjunction with information from the selective or specific bulk properties detectors in chromatography. More importantly, identification by mass spectrometry is universal for all chemical compounds as it relies on the mass-to-charge ratio, unlike some specific bulk detectors that may rely upon some unique properties relevant for just a handful of chemical species. The degree of insight about the sample identity from the MS data is typically proportional to the **mass accuracy** and the resolving power of the mass spectrometer used, which usually are proportional to the price of that instrument.

Unit-resolution mass spectrometers such as single quadrupole (SQ) mass spectrometers can rarely help in identification of a complete unknown without additional information from other techniques or sources. The SQ mass spectrometers typically can provide enough information for peak confirmation (as opposed to peak identification) to confirm or refute an existing hypothesis on the chromatographic peak identity. **High-resolution accurate mass (HRAM)** mass spectrometers such as TOF, Orbitrap, FT-ICR, and magnetic sector MS has a resolving power of more than tens of thousands and a mass accuracy approaching or exceeding ppm level. As a result, they can often unambiguously determine the **exact mass** of an ion (assuming known or single charge) and thus establish compound's molecular formula, elemental and isotopic composition. The exact mass parameter alone does not provide much insight into the molecular structure. However, when a unit mass or an exact mass are combined with the chromatographic retention time (assuming no co-elution and full chromatographic separation), the chromatographic peak confirmation or peak identification correspondingly can be established with very high degree of confidence, especially when an authentic reference compound is available or has been analyzed before under similar conditions. We will leave the notions of «isobaric interference» and of «mass defect», their degrees, and how to resolve them, outside the scope of this article.

Both the «unit mass» and the «exact mass» MS data can be further improved and refined with additional insight when combined with chemometrics restraints, data, and empirical rules (TAMI software from Aviv Analytical) as well as with an approach borrowed from the FT–IR vibrational optical spectroscopy on «profile data acquisition calibration» and applied to the mass spectrometry by Cerno Bioscience MassWorks™ software.

In addition to the unit mass or exact mass, most tandem and hybrid mass spectrometers can also provide **MS/MS** or **MS^n data**. Here, ions with an *m/z* value representative of the analyte are selected while all other *m/z* ions are eliminated. This is known as the precursor ion. Those ions are then excited, collided and/or reacted with a collision gas or reagent gas, and fragmented or chemically altered. These reactions under defined conditions are highly reproducible and are often characteristic of the structure. The resulting fragment ions (product ions) are then detected to produce an MS/MS or MS^2 spectrum. Or, some mass analyzers allow the product ions to be isolated and fragmented again to produce the MS^3 spectrum. Continuing this process results in what is known as MS^n. The collection of MS^n data from many or all ions of the analyte formed in the ion source, called **MS^n tree**, can serve for very precise structural identification, approaching the level of NMR. Specialized programs like Thermo Scientific™ Mass Frontier™ spectral interpretation software and Thermo Scientific™ Compound Discoverer™ small molecule identification software can greatly aid in both predicting (direct task) the MS tree from a structure drawn, and in analyzing (reverse task) of the experimental MS tree to deduce the unknown structural formula.

Although some types of the mass analyzers are nondestructive, all analytical mass spectrometers currently manufactured commercially are destructive detectors, unless designed and constructed specifically for preparative collection of the separated ions. The typical bottleneck of efficiency of most of the current mass spectrometers is their ionization source. Most of them, despite some notable recent improvements, remain largely inefficient. Yet, in the journey from the sample to knowledge, the technique of mass spectrometry remains one of the most insightful modern analytical techniques, along and on par with NMR.[2,3] The technique of the mass spectrometry «wastes» most of the sample introduced into the MS instrument, yet returns a great deal of sample knowledge and insights, including but not limited to the identification information.

The benefit of **quantitation** by mass spectrometry links, just like all other MS benefits, to the **selectivity at the molecular level**. The quantitation is rarely achieved by the MS alone and is currently performed mostly by hyphenated techniques or xC-MS, where x is either gas

chromatogarphy (GC), IC, or LC, or other «front end» separation technique, such as capillary electrophoresis (CE) or field-asymmetric ion mobility spectrometry (FAIMS). Chromatography provides separation of the chemical species at the bulk level by their different affinity to the chromatographic column's stationary phase, creating the first domain of the separation, the retention time domain or IC channel. Mass spectrometry separates the chemical species at the molecular level by their *m/z* ratio, creating the second domain of separation, the mass-to-charge domain, or mass domain (MS channel for short). In that sense, IC–MS closely resembles the IC × IC or 2D IC technique, with notable differences.

Unlike IC × IC, IC–MS provides the second separation domain that is **universal** and **sample information rich**. While the change in the set of analytes or the matrix often dictates a change in the selection of the second IC column in IC × IC, the MS adapts to those changes by simply adding another *m/z* to the acquisition method, or changing the SRM transition. At any point in time, the MS channel provides information that can be used for insight into the sample identity, purity, and presence of other co-eluting components or matrix interferences—none of which are readily available in the second domain of IC × IC. The common benefit shared between IC × IC and IC–MS is the increase in the **peak capacity** compared to the one-dimensional IC separation. Similar to 2D LC and 2D GC, 2D IC can be further hyphenated to become 2D IC–MS.[4,5] Examples when 2D IC was hyphenated simultaneously with both ESI–MS and ICP–MS, are known as well.[6]

When used for quantitation, the mass spectrometer as an analytical instrument acts as a **comparator**. In order to report the absolute mass or concentration of the substance, it needs a chemical standard to compare the signal from MS. The classical example of an analytical instrument that is not a comparator and, therefore, can be used for absolute quantitation without a chemical reference standard, are balances when used in a gravimetric analytical method – provided that we have other means to ensure the chemical identity of the substance being weighed out on those balances. (Note that there are some precise balances that are designed and constructed as weight comparators.) The need for a chemical reference standard for absolute quantitation by a mass spectrometer is due to a very large span of «ionization cross-sections» between different chemical species. This results in very large differences in ionization efficiencies and, therefore, the resulting signal in the MS channel used for quantitation.

The currently accepted «gold standard» of **targeted quantitation** is by a triple quadrupole MS (often abbreviated as QqQ) coupled to a front end chromatograph: GC–MS/MS, LC–MS/MS, and IC–MS/MS. The «QqQ» denotes two analyzing quadrupoles (denoted by the upper-case Qs) and either a quadrupole (lower-case q) or some other multipole (e.g. hexapole or octapole) collision cell. This concatenation of relatively inexpensive quadrupole mass analyzers allows a synergistic gain in selectivity via the MS/MS experiments, where the first quadrupole analyzer (Q1) selects the precursor ion(s), which collide with inert gas, fragment in the collision cell (q), and the resulting product ions are analyzed in the last quadrupole (Q3). The analyst then selects and monitors the most characteristic and interference-free pair of ions: the precursor ion and the product ion, together called a **«transition»**. This transition represents the gas-phase chemical reaction that occurs during the flight of the precursor ion through the mass spectrometer, sometimes also referred to as **«selected reaction monitoring» (SRM) or «multiple reaction monitoring» (MRM)**, since the analyzing quadrupoles can switch very quickly (on the order of milliseconds) from one *m/z* ion to another. The terms SRM, MRM, and MS/MS refer to exactly the same process that is responsible for increased selectivity of the triple quadrupole MS at the molecular level. To achieve this enhanced selectivity, the analyst must make the selection of the SRM transitions for the MS/MS experiments, thus it is only possible to take advantage of it for **known analytes** (targeted quantitation) after MS/MS method development. If we know what analytes we want to detect and quantify, we perform targeted quantitation with a triple quadrupole MS. The triple quadrupole MS is not the quantitation technique of choice for the analysis of complete unknowns though.

For **untargeted quantitation**, or analysis of unknown compounds (unknowns), we need high-resolution accurate mass (HRAM) mass spectrometers that can operate at sufficiently high resolving power (typically 60,000 FWHM or above) to identify unknowns, and with sufficiently high speed (typically defined by the width of the chromatographic peaks) in order to perform quantitation with enough data points across the chromatographic peak. It is important to note that HRAM is fully capable of targeted quantitation as well, yet the higher price of those instruments often dictates their choice only when their price is justified by the application need (i.e. untargeted analysis). The method development for quantitation with an HRAM mass spectrometer is simpler than that with a

triple quadrupole. The higher selectivity of HRAM allows for a **simplified sample preparation**, sometimes reduced to a simple dilution of the sample before injecting it into the «front end» ion chromatograph. Because of these characteristics, we speculate that in the future HRAM mass spectrometers become more affordable and widespread, at which point all the quantitations currently performed by triple quadrupoles, migrate to HRAM methods with higher confidence in the data, simplified method development and sample preparation, higher matrix tolerance, and similar or better limits of detection.

As compared to bulk-level chromatographic detectors, a single quadrupole mass spectrometer offers significantly higher selectivity on the molecular level. For example, a **single quadrupole** (such as ISQ EC Single Quadrupole Mass Spectrometer) coupled to an ion chromatograph offers a number of benefits to the analytical chemists. Chromatographic co-elutions can be resolved in the MS domain rather than in the retention time domain. In other words, even if two chromatographic peaks completely co-elute, we can still identify and quantitate them individually as long as they form ions with different *m/z* values. That is, the single quadrupole MS can resolve non-isobaric co-elutions because they have different *m/z*.

Therefore, **automatic peak integration** becomes much more reliable, even if the peaks are not symmetric and show some fronting, tailing, shoulders, and even complete overlap and co-elution. Thus, adding MS to IC makes the chromatographic method development a more forgiving task. The technique of IC–MS increases the matrix variability tolerance, since the modern software (e.g. Chromeleon™ software) can adjust the auto integration peak window based on the *m/z* value of the targeted analyte. If the matrix change causes the retention time to drift somewhat, the quantitation will still be performed correctly, without the need of manual peak integration by the analyst. This benefit alone may reduce the number of repeat injections and time spent on data review in high throughput laboratories and therefore justify the upgrade from stand-alone IC to IC–MS.

The detector(s) of any mass spectrometer always detects as signal, directly or indirectly, the flux of charged particles with a particular *m/z* ratio segregated either in time (most often) or in space. Thus, the quantitation in IC–MS is always performed on the selected *m/z* signal(s) (chosen by the analyst) or on a linear combination thereof. For example, if bromide, bromate, and perchlorate anions have to be quantitated in drinking water with a single quadrupole IC–MS, our choices of quantitation MS channels (called **selected ion monitoring** or **SIM**) would be as follows in Table 4. Depending on the goal (for example, to achieve the lowest limit of detection), the analyst may choose to calibrate and quantitate on the combination of signals from both $^{79}Br^-$ and $^{81}Br^-$, which nature provides to us in about equal abundance. In another analytical case, the analyst may choose only one particular isotopologue to quantitate upon. For example, when drinking water is tested for perchlorate, it is usually *m/z* 99 that is chosen as the **quantifying ion**, while *m/z* 101 is chosen to be the **confirming ion**. The choice of the quantifying ion is clear due to the higher abundance of the chlorine-35 isotope in nature, providing a higher signal intensity from the perchlorate isotopologue $^{35}Cl^{16}O_4^-$. The need for a confirming ion stems from the fact that there could potentially be other compounds (e.g. $H^{34}SO_4^-$) present with *m/z* 99, while the chance that these potentially interfering compounds would also have *m/z* 101 at the same time, is much less probable. Note that not only do these potentially interfering compounds need to be actually present to affect our targeted quantitation, but they also would have to possess exactly the same chromatographic retention time. Thus, the very notion and the practice of confirming ion utilization in IC–MS methods reflects the advantage (rather than disadvantage) and the more selective nature of IC–MS versus stand-alone IC.

The IC–MS **method flexibility** is also superior to that of stand-alone IC. In order to change the selectivity of the IC–MS method, the analyst only needs to choose a different MS channel. There is usually an alternative *m/z* that is often available. For example, if the matrix of a particular sample interferes with the quantifying ion of perchlorate at *m/z* 99, the analyst can simply switch and quantitate on *m/z* 101 instead. There are also multiple approaches to create different *m/z* quantifying ions: either through complexation and wet chemistry at the stage of the sample preparation (e.g. carbohydrate complexation with lithium cations), post-column derivatization or complexation, in-source reaction (chemical reactions occurring in the ESI ionization source of the mass spectrometer), or MS/MS fragmentation reactions with inert collision gas(es) or with reactive gases, such as ammonia.

Table 4. Isotopologues of bromide, bromate and perchlorate.

Species	Exact mass, amu	Nominal *m/z*	Relative abundance, %
$^{79}Br^-$	78.9177885	79	50.69
$^{81}Br^-$	80.9157420	81	49.31
$^{79}Br^{16}O_3^-$	126.9025324	127	50.69
$^{81}Br^{16}O_3^-$	128.9004858	129	49.31
$^{35}Cl^{16}O_4^-$	98.9479626	99	75.76
$^{37}Cl^{16}O_4^-$	100.9450124	101	24.24

Note that the average molar mass of perchlorate ClO_4^- (99.4320 amu) calculated from the Dmitry Mendeleev's Periodic Table of Elements that is used for analytical chemistry gravimetrical calculations is *Not* observed in IC–MS, for it does not correspond to any actual molecular species separated and detected by the mass spectrometer analyzer/detector. This is yet another reminder that mass spectrometry is an analytical technique that is selective at the molecular level rather than the bulk level of chromatographic detectors.

The additional criteria often employed in IC–MS quantitation methods that both ensures accurate identification of the target compound and reduces the risk of false positive contribution to the quantitation value from potentially interfering components is the **ion ratio**. For example, if the analyst had chosen *m/z* 127 for the quantitation of bromate in drinking water, and *m/z* 129 is the confirming ion, the additional criteria of ion ratio between the two masses being close to 50.69:49.31 ≈ 1:1 would reduce the risk of any chemical species other than bromate to contribute to the quantitatively reported bromate value.

It is useful to remember twenty-one elements that are naturally mononuclidic in the air, water, and in the upper crust of the earth: ^{9}Be, ^{19}F, ^{23}Na, ^{27}Al, ^{31}P, ^{45}Sc, ^{55}Mn, ^{59}Co, ^{75}As, ^{89}Y, ^{93}Nb, ^{103}Rh, ^{127}I, ^{133}Cs, ^{141}Pr, ^{159}Tb, ^{165}Ho, ^{169}Tm, ^{197}Au, ^{209}Bi, ^{231}Pa. The elements of extraterrestrial origin (solar wind, cosmic dust, meteorites), the artificially synthesized elements, the elements in the upper atmosphere, and the elements in the lower crust/mantle may and very often do exemplify different isotopic abundances and may not be mononuclidic.

Precisely because mass spectrometers can discern the exact molecular species (selectivity at the molecular level), when they are analyzed in the ionized forms, one of the most precise method of quantitation by IC–MS is that which employs **«isotopically labelled internal standards»** (ILIS). Typically, stable isotope(s) not found in nature at high abundances are incorporated into the molecule of interest to make it highly unlikely to be found in nature as the contaminant that we would be aiming to quantify. For example, in the analysis of perchlorate, the artificially synthesized perchlorate isotopologue $^{35}Cl^{18}O_4^-$ (nominal *m/z* 107) enriched with ^{18}O (natural abundance 0.205%) rather than the predominant ^{16}O (abundance 99.757%) is utilized as the isotopically labelled internal standard, which greatly increases both the accuracy and the limit of detection of the IC–MS quantitation method. The isotopically labelled compounds used as internal standards tend to be rather expensive and are necessarily consumed during the analysis, imposing an ongoing operational burden to the routine testing laboratories. Thus, the utilization of ILIS is a judgment call of the analyst and is dictated by the regulated method, the complexity of the analyte(s), or the matrix, or both.

Let's consider another quantitation example: the analysis of haloacetic acids (HAAs) by IC–MS. The analytes are a set of closely related and chemically similar compounds (often called congeners) that can form via numerous pathways, including the water disinfection with chlorine or chlorine oxide. All HAAs have higher acidity than the unsubstituted acetic acid and, therefore, reversed phase HPLC columns poorly retain such polar analytes, while an ion-exchange column specifically designed for this purpose can separate them. The compounds have different toxicity levels and thus need to be quantified separately and accurately. This is a common theme in many other analytically challenging cases, e.g., analysis of the dioxin congeners. Although the matrix is typically drinking water, a seemingly simple matrix, the presence of other ions such as chloride and sulfate at high concentrations interfere with ionization of HAAs at trace level. This is called a **matrix effect** in IC–MS. Ion suppression or (less often) ionization enhancement of the small concentrations of the target analyte in presence of high or sometimes similar concentrations of other components eluting at the same time as the analyte. Other hyphenated techniques such as LC–MS and GC–MS may also suffer from the matrix effects.[7,8,9]

This is where the ability of the «front end» IC to physically separate the analytes in the chromatographic column one from another and each analyte from the matrix ions, becomes indispensable. The approach (e.g. U.S. EPA Method 557) is to include chromatographic column effluent diversion windows to prevent high concentrations

of the interfering ions from tap water from entering the MS ionization source. This is achieved with a diverter valve installed on the ion chromatograph. However, even with this approach, the chemical noise present in the column effluent at every point in time requires a mass spectrometer with a higher degree of selectivity than a single quadrupole. In order to achieve sub-ppb (triple digit ng/L) limits of detection for all individual HAAs, the higher-selectivity technique of IC–MS/MS is required to be used together with a few isotopically labelled internal standards. Additional synergy between the IC and MS can be achieved when the diverter valve is controlled automatically when the CD detects high conductivity (e.g. > 50 micro Siemens).

In its simplest, IC–MS/MS can be performed by either an ion trap (MS/MS in time) or a triple quad (MS/MS in space), with the triple quadrupole being currently the predominant choice due to the QqQ analyzer's high speed of analysis. This is especially important with the closely eluting and often co-eluting HAA analytes. Many fragments in the mass spectra of the HAAs are common and shared. For example, monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), and trichloroacetic acid (TCAA) will all have a chloride ion amongst their fragments. This is where the power of selected reaction monitoring (SRM) of the triple quadrupole versus the single ion monitoring (SIM) of the single quadrupole comes into play. Even if two analytes of interest, TCAA and bromodichloroacetic acid (BDCAA), have ions with **the same nominal mass** *m/z* 163, the technique of IC–MS/MS can still distinguish between them and quantify them separately and accurately via two different SRM transitions, denoted as 163 → 119 for TCAA and 163 → 81 for BDCAA. Because TCAA does not have any bromine in its structure, the transition 163 → 81 is specific for the bromine-containing BDCAA.

There are classes of chemical compounds that have an identical molecular formula, and therefore are **isobaric**. Isobaric (meaning «equal weight») compounds have the same nominal mass. One of such classes of compounds that has a particular importance in our life is the carbohydrates. Let's take D-glucose as an example, the monosaccharide that circulates in our blood. It has the molecular formula $C_6H_{12}O_6$, with nominal mass of 180. There are four stereo centers in the molecule of glucose, that is there are 2^4 stereoisomers possible, and all sixteen are known. Fourteen of them are diastereomers. In solution, each one of the sixteen stereoisomers can exist in two cyclic, hemiacetal, pyranose forms: each one being an anomer of another. The diastereomers (and anomers at low temperature) can potentially be separated on non-chiral chromatographic stationary phases, yet the structural differences between them are so miniscule that this is indeed a challenging task. Not surprisingly, carbohydrates as a class are also a challenge to be analyzed by mass spectrometry. Not only are they isobaric, but also due to very similar chemical structures, they rarely have different fragment ions in their mass spectra. To identify and quantify carbohydrates, one needs to employ the technique of IC–HRAM–MS with a mass analyzer that possess resolving power 60,000 FWHM or above, such as an Orbitrap mass spectrometer. This is where the synergistic resolution of carbohydrates in the retention time domain of ion chromatography compliments the high-resolution of MS in the *m/z* domain.

For comprehensive review of ion chromatography, please refer to the «Handbook of Ion Chromatography» by Joachim Weiss, which has been published in three volumes in its Fourth Edition in the year 2016, ISBN 3527329285 and Thermo Fisher Scientific part number 1R120400-0050. This is comprehensive treatment of the subject, covering the principles, instrumentation, methods, and applications, including IC–MS.

For a good technical introduction to the area of mass spectrometry, consult the «Mass Spectrometry, A Textbook», by Jürgen Gross, published in its Third Edition in 2017, ISBN 3319543970 and Thermo Fisher Scientific part number 1R120400-0040.

In-depth discussion of the topic of xC–MS quantitation can be found in the book: «Trace Quantitative Analysis by Mass Spectrometry», by Robert Boyd, Cecilia Basic, and Robert Bethem, published in 2008, ISBN 0470057718.

thermo scientific

References

1. Heiko Hayen, HILIC—Polare Verbindungen trennen. Nachrichten aus der Chemie, 2010, 58 (4), 461–465.
2. D.D. Marshall, R. Powers, Beyond the Paradigm: Combining Mass Spectrometry and Nuclear Magnetic Resonance for Metabolomics, Progress in Nuclear Magnetic Resonance Spectroscopy (2017), DOI: http://dx.doi.org/10.1016/j.pnmrs.2017.01.001.
3. Combining NMR and MS with Chemical Derivatization for Absolute Quantification with Reduced Matrix Effects (2019), DOI: http://dx.doi.org/10.1021/acs.analchem.8b05611.
4. Miller, M, Schnute, W Direct Determination of Small Organic Acids in Sea Water by IC-MS. [Internet]. [cited 2016 July]. Available from: https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-1000-IC-MS-Organic-Acids-Sea-Water-AN70185-EN.pdf.
5. Unbiased Measurement of Phosphate and Phosphorus Speciation in Surface Waters, Environ. Sci. Technol. 2019, 53 (2), 820-828, DOI: 10.1021/acs.est.8b05089.
6. Quantification of ionic organo(fluoro)phosphates in decomposed lithium battery electrolytes RSC Adv., 2017, 7, 39314.
7. 2005 Paul Taylor Matrix effects: The Achilles heel of quantitative high-performance liquid chromatography–electrospray–tandem mass spectrometry, Clinical Biochemistry 38 (2005) 328–334.
8. Anneli Kruve et al. Tutorial review on validation of liquid chromatography mass spectrometry methods: Part I, Analytica Chimica Acta DOI:10.1016/j.aca.2015.02.017.
9. Jean-François Bienvenu et al. Standardized Procedure for the Simultaneous Determination of the Matrix Effect, Recovery, Process Efficiency, and Internal Standard Association. Anal. Chem. 2017. DOI: 10.1021/acs.analchem.7b01383.

Find out more at **thermofisher.com/icms**

ThermoFisher SCIENTIFIC