Liquid chromatography mass spectrometry (LC/MS) techniques are often used to analyze compounds that are non-volatile and thermally unstable. Electrospray ionization (ESI) can continuously evaporate liquid solvent and has become an important technique for use as an interface for LC/MS techniques. As liquid solutions flow out of the separation column, they are directly transformed into gaseous ionized molecules by applying a high voltage to the solution, creating a fine spray of highly charged droplets. Simultaneously, dry nitrogen gas and heat are also applied to the charged droplets to evaporate the liquid solvent at atmospheric pressure [1-3].

ESI is a soft ionization process that allows observation of intact molecules that are multiple or single charged. The number of charges on a particular molecule depends on several factors such as structure, size, composition, solvents used in LC, and instrument parameters. In general, single, double, or triple charged molecules are produced for small molecules (< 2000 Da), while multiple charges are produced for large molecules (> 2000 Da). In addition, the ability to produce multiple charged molecular ions makes it possible to observe very large molecules (e.g. a large variety of polymers, proteins, and DNA fragments) using an instrument with a relatively small mass range. It also allows sensitive detection of single charged low-molecular-weight molecules (e.g., amino acids, drugs and metabolites) [2]. Furthermore, molecular ionization processes can result in positively and negatively charged ions. For example, protonated, ammonium, and alkali adducts are generally observed in the mass spectra for positively charged molecules, while deprotonated molecules are observed for negatively charged compounds [4].

Generally, positive ion detection is used for molecules with functional groups that readily accept a proton (such as amines), and negative ion detection is used for molecules with functional groups that readily lose a proton (such as carboxylic acids). Although most molecules will be initially ionized in the positive ion mode, successful analysis cannot always be accomplished using the positive ion mode. Therefore, it is often necessary to use the negative ion mode, which can be advantageous since it sometimes generates less chemical background noise compared to positive ion mode [3].

To obtain structural information, common types of mass spectrometers are used, such as the triple quadrupole mass spectrometer (QqQ) and the (linear) iontrap mass spectrometer (ITMS) [5]. QqQ is a powerful technique for confirmation and quantitative analysis of individual compounds using the selected reaction monitoring mode (SRM). In this mode, the molecular ion of interest (precursor ion) is selected in the first quadrupole and allowed to react with a collision gas in the collision cell. The precursor ions then undergo fragmentation, resulting in product ions that are related to the molecular structure of the precursor ions. Finally, the product ions are selected in the third quadrupole and monitored, providing structural information of the molecular ions [1, 4, 5].

This provides reliable confirmation and allows selective and sensitive quantitation of the molecule of interest. Information is available for a broad linear dynamic range with high accuracy and reproducibility, especially at low...
concentration levels compared to other techniques [5]. Furthermore, the high selectivity of the SRM mode results in less interference from co-eluting matrix compounds. Therefore, less compound separation and less stringent sample preparation are necessary. In the case of accurate enrichment analysis of stable isotopes, the mass-unit resolution is not sufficient to separate isotopic peaks. For accurate determination of stable isotope enrichment, the resolution should be increased, which compromises sensitivity. Additionally, the LC/MS operating in the SIM (selected ion monitoring) mode may also not be sufficient for accurate enrichment analysis due to interfering matrix compounds influencing the tracer-to-tracee ratio (TTR), which represents the ratio between the labeled (tracer) and the unlabeled compound (tracee). Therefore, the SRM mode should be used to improve the accuracy, selectivity and sensitivity of the measurement.

However, fragmentation of the tracer can result in loss of the stable isotope, so understanding the fragmentation pattern is important for using fragments containing the stable isotope. Indeed, the mass difference of the precursor ions of the tracer and tracee is sufficient in the SRM mode for enrichment analysis, but it is advisable to choose a fragment containing the stable isotope. Applications have been reported for which LC/MS-MS is a good alternative for gas chromatography coupled to combustion isotope ratio mass spectrometry (GC/C/IRMS) [6-8] when small sample volumes are available, but they have only concerned stable isotope tracers that are well separated from the natural isotopic pattern. Meesters et al. [9] described a detection limit of 0.01% TTR for amino acid tracers [L-ring-d5]-phenylalanine, [L-ring-d4]-tyrosine, and [L-ring-d2]-tyrosine in human plasma using LC/MS-MS.

The (linear) ITMS is another common type of mass spectrometer that is often used for analysis. It consists of a quadrupole or a spherical trap that capture ions. It can also be used as a selective mass filter [10-12]. ITMS differs from QqQ instruments by the pulsing of the mass analyzer rather than continually entering the mass analyzer. The quadrupole or spherical trap captures ions in a stable oscillating trajectory that depends on the potential and the m/z ratio of the molecular ions. During detection, the potential is altered to produce instabilities in the ion trajectories, resulting in ejection of the ions in order of increasing m/z ratio. The ITMS is also capable of triple quadrupole-like fragmentation. The precursor ion is selected in the trap, where an inert gas is introduced for fragmentation. In the next step, the product ions are ejected from the trap for detection. Additionally, fragmentation of the product ions can be continued for several times (MS^n) by keeping the product ions inside the trap and repeating the fragmentation procedure. In the case of enrichment analysis, the full-scan and SIM mode are commonly used in ITMS. However, for accurate enrichment analysis, the best results are obtained by using the full-scan mode rather than the SIM mode. In the SIM mode, ions are isolated in a waveform, which means that focus is on ions in the center of the selected mass range. Since the SIM mode is designed for the isolation of a narrow range of ions or a single mass, the various isotopes should be measured individually to determine TTR’s. On the other hand, in the full-scan mode a range of ions are collected and detected regardless of the ions at the extremes of the selected mass range. As a result, in the full-scan mode, the ions are collected and detected in a single step, resulting in more accurate TTRs.

To obtain accurate results with ITMS analysis, the full-scan mode in combination with enhanced resolution (e.g., zoom mode) is recommended for analysis. Particularly, the ITMS can operate with enhanced resolution, which is particularly beneficial for accurate enrichment analysis since isotopic peaks are baseline-separated. Additionally, the enhanced resolution may also be beneficial for separation of co-eluting matrix interferences. Since high resolution is not always sufficient for separation of matrix interferences, the ITMS can also operate in the SRM mode to improve the selectivity of the measurement.

In ITMS, fragmentation of fragile ions during the isolation of the precursor ion in the SRM mode results in loss of isolated ion intensity. To obtain adequate ion intensity in the SRM mode of fragile precursor ions, a wider ion isolation width is required. However, the increased isolation width significantly diminishes the selectivity of the SRM transitions, which is a serious problem for samples with complex matrices [13]. In ITMS analysis, it is also important to understand the fragmentation pattern in order to use fragments containing the stable isotope. Several applications have been reported related to stable isotope enrichment of amino acids in plasma with ITMS analysis using enhanced resolution [14, 15]. Derivatization of amino acids enables estimation of isotopic enrichments down to 0.005% TTR [14].

In conclusion, resolution is an important issue in ITMS as well as QqQ analysis since the isotopic peaks should be baseline-separated for accurate isotopic enrichment. In most cases, the sensitivity is compromised with both techniques to improve the resolution [5]. In general, QqQ provides a higher sensitivity (at least 20-fold), better linear dynamic range (QqQ 10^5–10^7; ITMS 10^4–10^6), and better repeatability (QqQ (5–9%); ITMS (12–16%)). In
contrast, ITMS has better resolution (QqQ varying from 0.07 to 1 Δm/Δz, ITMS varying from 0.05 to 0.1 Δm/Δz) [5]. Although the sensitivity of QqQ in the SRM and SIM modes is much better, in the full-scan mode, ITMS provides higher sensitivity. Since most ITMSs use automatic gain control (AGC), they are sensitive for co-eluting peaks because the ion storage times are influenced by co-eluting substances, which could affect the sensitivity. Consequently, in ITMS, chromatography could play a crucial role in some cases by separation of co-eluting matrix interferences with the analyte of interest. To obtain accurate isotopic measurements in QqQ analysis, the SRM mode should be used to improve the accuracy, selectivity, and sensitivity of the measurement. The ITMS can also operate in the SRM mode to improve the selectivity of the measurement, but it is well known that fragmentation of fragile ions in ITMS results in loss of isolated ion intensity. To obtain adequate ion intensity in the SRM mode, a wider isolation width is required. Applications have been reported in which LC/MS-MS is a good alternative for GC/C/IRMS [6, 7], and this technique may be useful for small sample volumes and in the case of stable isotope tracers that are well separated from the natural isotopic distribution. However, there are also high-resolution QqQ analyzers on the market that might be more suitable for stable isotope enrichment analysis. In other cases, when the resolution plays an important role, ITMS is the technique of choice.

References

Citation:
Open Access and Copyright:
©2015 OOSTERINK JE and SCHIERBEEK H. This article is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY) which permits any use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

Funding/Manuscript writing assistance:
The authors have no financial support or funding to report and they also declare that no writing assistance was utilized in the production of this article.

Competing interest:
The authors have declared that no competing interest exist.