Chapter

Quantitative and Qualitative LC-High-Resolution MS: The Technological and Biological Reasons for a Shift of Paradigm

Bertrand Rochat

Abstract

Today, high-resolution mass spectrometry (HRMS: Q-TOF-MS, Orbitrap-MS) shows sensitive and reliable quantifications of a large variety of compounds while acquiring in high-resolution full-scan mode. Interestingly, HRMS shows equal quantitative performance than triple-quadrupole-MS (QQQ-MS), which is the MS technology traditionally used for quantification. But, in contrast to QQQ-MS that performs "narrow-minded" ion transitions (targeted prior determination), analysis using HRMS can record HR-full scan that detects virtually all ions (e.g., from m/z = 80 to 1000) and gives a global picture of what is in the biological sample (diagnostic screening). This is more and more seen as a key advantage because on top of targeted and quantitative analyses, many other routine or research determinations can be performed such as qualitative (identification), simultaneous quantitative/qualitative (quan/qual), and omics (untargeted) assays. The high versatility and performance of most actual HRMS instruments placed them as new gold standards in LC-MS analysis. Indeed, only HRMS can answer new analytical requests from systems biology and personalized medicine requesting more holistic approaches with untargeted analyses (e.g., proteomics and metabolomics). In the light of the new HRMS-based paradigm, concrete examples revealing quantitative, qualitative, simultaneous quan/qual, and omics capabilities of HRMS in the context of routine and research analyses will be given.

Keywords: diagnostic, HRMS, high resolution, LC-MS, liquid chromatography, mass spectrometry, metabolomics, Orbitrap-MS, proteomics, quantitative, qualitative, routine, screening, Q-TOF-MS, triple-quadrupole-MS, untargeted

1. Introduction: analytical tools and the understanding of the biology

In life sciences, liquid chromatography coupled to mass spectrometry (LC-MS) is considered as a tool to measure molecules in biological samples. In clinical and toxicology labs, LC-MS determinations are quantitative and routinely performed and usually measured one or a few compounds [1–4]. These analyses are strongly driven by medical knowledge as well as the understanding of disease biology. Our understanding of the biology is, to a large extent, related to the available analytical tools. On the other hand, new concepts in life sciences can promote

the development of new analytical tools. The actual understanding of biological systems is presented here below. It will help to put into perspective the actual and future needs for LC-MS analysis and MS technology.

Today, we can draw three main bricks of life, genome, proteome, and metabolome (**Figure 1**), respectively, and to determine nucleic acids, proteins, and metabolites, there are various analytical tools in which, for the last two bricks, mass spectrometry (MS) plays a central role. Whereas **Figure 1** describes three equal bricks of life, the analytical tools have somehow driven DNA far ahead in terms of grants and publications. Indeed, **Figure 2** depicts the number of publications collected in PubMed database for the last 20 years in relation to these three bricks, "genome, proteome, or metabolome" and "patients" as key words. **Figure 2** reveals that the number of publications related to the genome is much higher (log scale) than for the proteome and metabolome. It underscores the efficiency of DNA chips (high-throughput sequencing) as analytical tools. **Figure 2** also reveals the higher number of publication for the proteome than for the metabolome. A steady state between these three bricks of life is expected to come in a near future because genome, proteome, and metabolome have complementary information and because recent MS technologies with improved analytical capabilities, have been launched during this last decade. These recent MS are high-resolution mass spectrometers (HRMS) and allow cheap targeted and omics/global approaches. They should promote proteome and metabolome determinations not only in research but also in routine laboratories for diagnosis purposes (e.g., clinical and toxicology labs).

The main advantages of DNA sequencing over the determination of proteins and metabolites are that (1) DNA can be multiplied (PCR), (2) the DNA sequence is (roughly) constant over the entire life, and (3) the analysis has become really affordable. On the other hand, the great advantage of the determination of the proteome and/or the metabolome over the genome is the integration of all types of interactions and memories (e.g., age) that the living system has with its environment (**Figure 3**) [5]. This last point is crucial and supports an increasing need for individual's proteome and metabolome determination.

In addition, the concepts of "long tail disease" and "tyranny of the average" have recently emerged (**Figure 4**) [6, 7]. These concepts underscore the high number

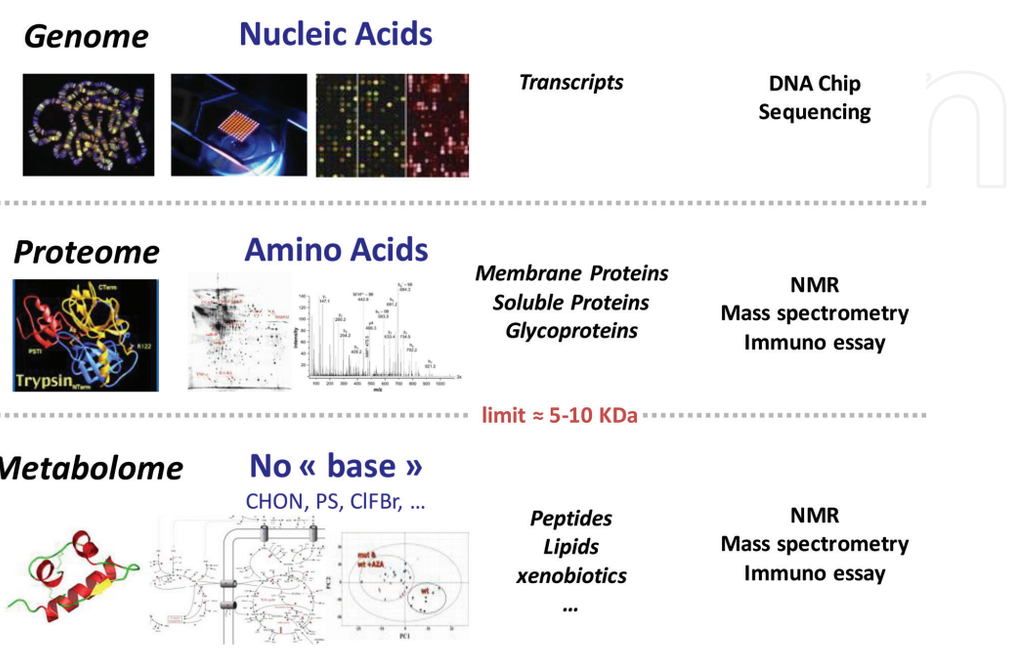

Figure 1.
The three mains of life, their bases, entities, and analytical tools.

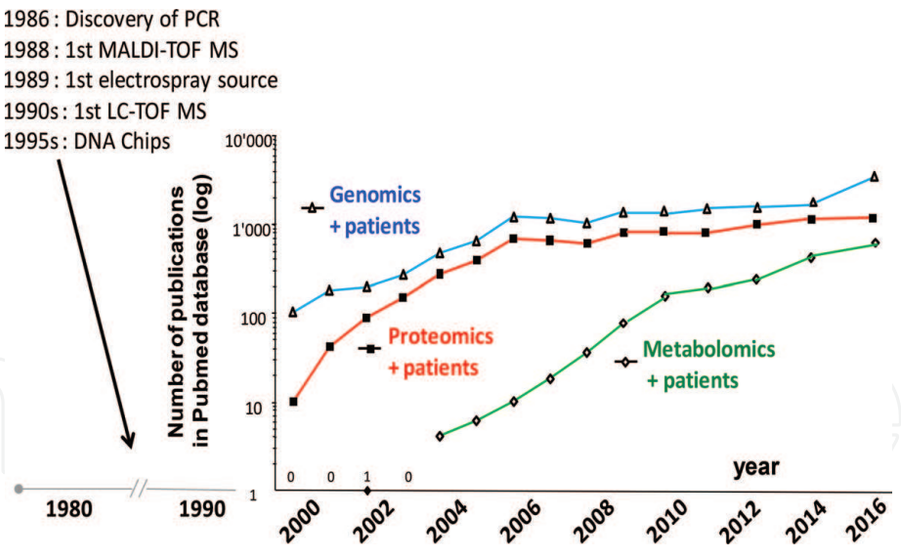

Figure 2.
Number of publications in PubMed database in relation to three keywords, "genomics," "proteomics," and "metabolomics," associated to "patients" over the last two decades.

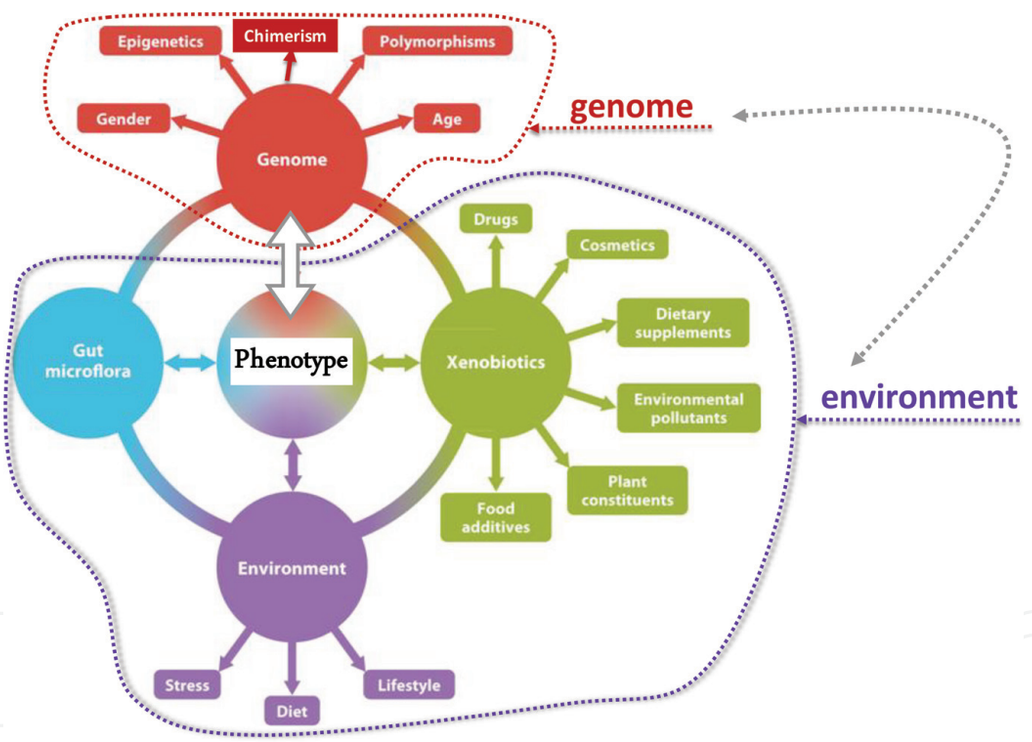

Figure 3.
System biology and its holistic approach. The phenotype of an individual is the results of the interaction and memories of the genome and the environment. It sustains that a global approach is valuable in personalized medicine (adapted from [5]*).*

of low-prevalence diseases. Low-prevalence diseases represent a significant part of many diseases that are wrongly considered as homogenous (etiology or molecular mechanism of the disease) and would be diagnosed with many different biomarkers. Low-prevalence (sub-)diseases suggest that there is a need to determine more globally what is in the patient's sample (the proteome and/or the metabolome) in order to find unexpected outsider molecules that will be at a low frequency but with a high significance as biomarkers. Eventually, these concepts promote medical doctors and bioanalysts to favor more holistic approaches for a higher consideration of individual (variations limiting the tyranny of the average) [6, 7].

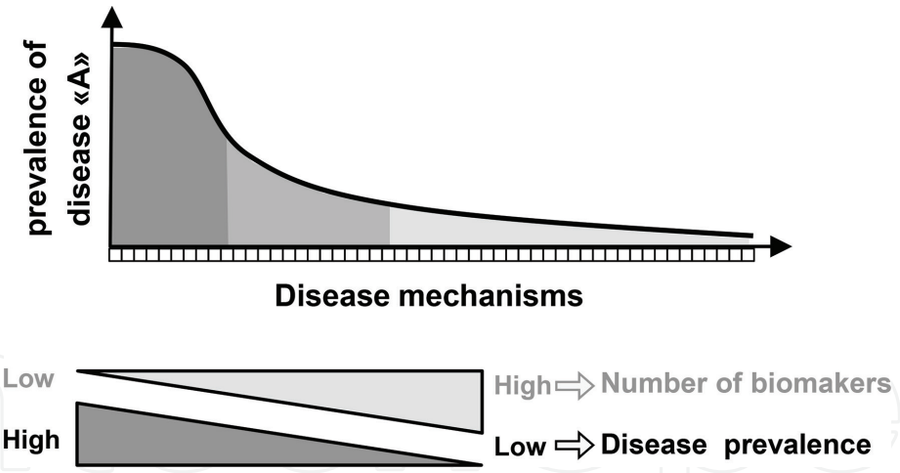

Figure 4.
Prevalence of a disease "A" against disease mechanisms. The figure depicts a long tail curve suggesting that, beside the frequent and few molecular mechanisms or origins of the disease (left part of the curve), there are many low-prevalence subcategories of the "same" disease (right part of the curve). In this scenario, the determination of a few usual biomarkers can be useless for many patients. The potential high number of biomarkers with low prevalence but high significance suggests that untargeted acquisition and untargeted data treatment have their place in biomedical analyses.

These actual concepts in life sciences have become crucial for understanding the complexity of biological systems and for delivering better personalized diagnostics and medicine. It promotes the LC-MS instruments capable to record "global view" in one affordable analytical shot [8, 9].

2. LC-MS technologies

This can be useful to recall that liquid chromatography coupled to mass spectrometry (LC-MS) is composed of three parts: (1) the (U)HPLC system, (2) the atmospheric pressure ion (API) source (most frequently the API source type is an "electrospray"), and (3) the mass analyzer (**Figure 5**) [10]. The ion source is an interface between the liquid and the gas phase, where the mobile phase is vaporized, leaving the ions in the gas phase and ready to enter in the MS. Even if the second and third parts are fully integrated, the ionization chamber can rapidly be changed by another source that is designed for different flow rates or modes of ionization. The LC-MS analysis records chromatograms where *m/z* values (ions = mass over charge ratios) are detected (relative intensity) and depicted over time (**Figure 5**). Ions are detected as positive or negative adducts: usually $+H^+$ but also $+Na^+$, K^+, $NH4^+$, and $-[H^+]^-$. There are three main MS technologies (ion trap MS would be the fourth but it is not described here) that can be divided in low and high mass resolution instruments (**Figure 6**). There are triple-quadrupole (QQQ-MS), (quadrupole-)*time-of-flight*-MS (TOF-MS), and (quadrupole-) Orbitrap-MS (Orbi-MS; e.g., Q Exactive).

Most frequently, mass resolution (R) is defined as $R = m/\Delta m_{FWHM}$, where [m] and Δm_{FWHM} correspond to the *m/z* of the ion considered and the distribution of *m/z* at full-width half-maximum of the peak height (FWHM), respectively (Δm_{FWHM} units are Da, u, or Th; **Figure 7**) [11]. Mass accuracy is defined as the delta between the theoretical and measured *m/z* and is given in mDa (u or mTh) or in ppm [11]. Accurate mass determination allows to extract ions with narrower mass-extraction-window (MEW) in order to construct extracted-ion chromatograms (XIC) [12].

Low- and high-resolution mass spectrometers are defined with an R value below or above 10,000, respectively. Low-resolution MS (LRMS) are QQQ-MS, whereas

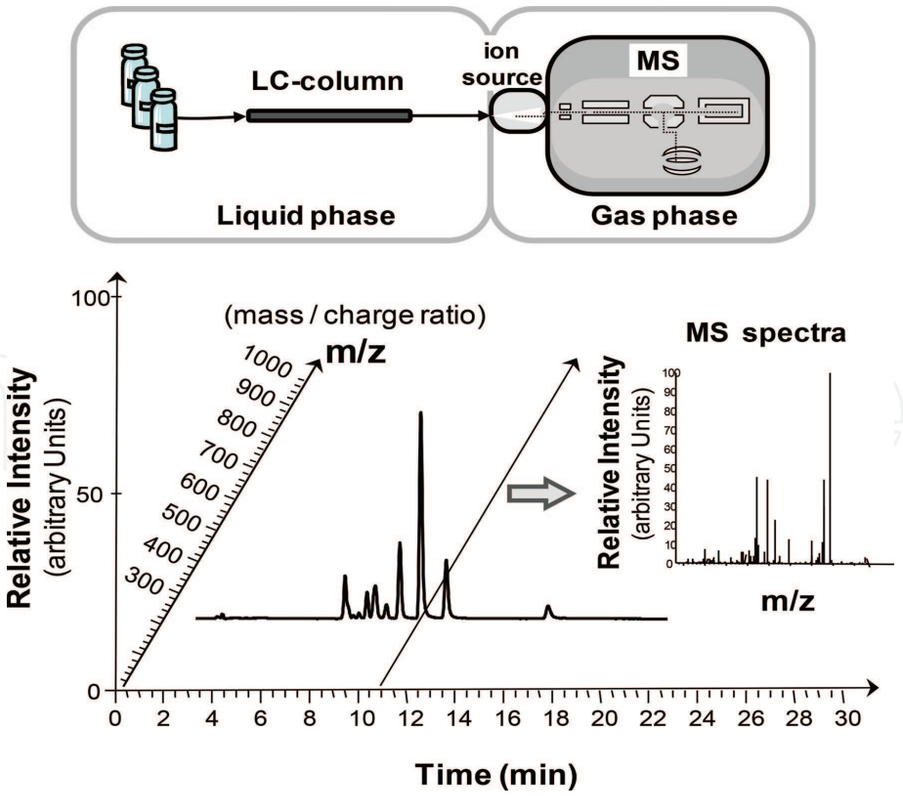

Figure 5.
LC-MS analysis. LC-MS systems are composed of a LC part, an ion source, and a MS analyzer. LC-MS analysis is defined mainly by a retention time, a relative intensity (arbitrary units), and the ions detected (m/z, mass over charge ratio).

high-resolution MS (HRMS) are TOF-MS and Orbi-MS instruments. It is worth to note that R is not a constant value over the mass range (**Figure 8**) [12]. R values are usually given at *m/z* = 200 and 400 for Orbi-MS and TOF-MS, respectively. Today, R

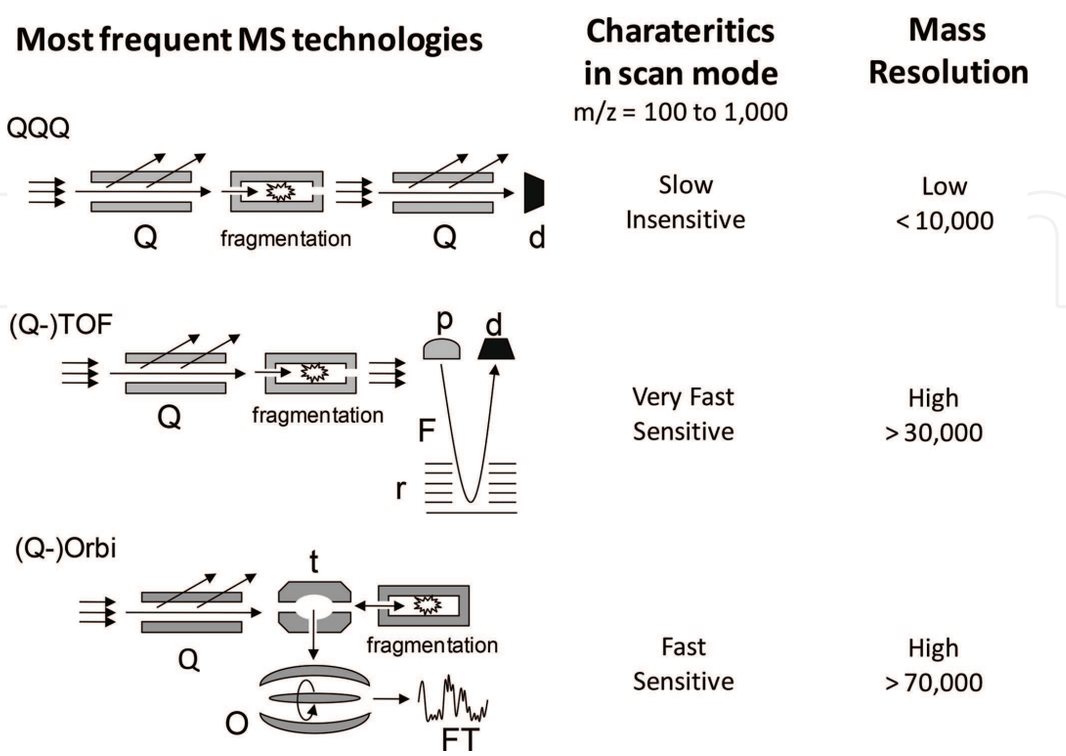

Figure 6.
The three most frequent MS technologies and their key characteristics for scanning rate and mass resolution (adapted from [7]).

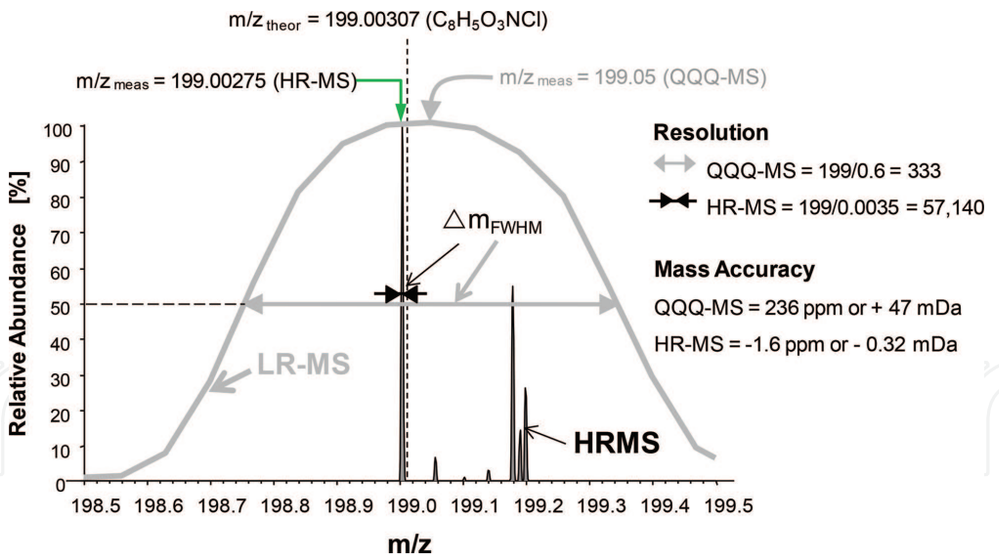

Figure 7.
Sketch showing what are the mass resolution and the mass accuracy. Typical mass resolution and mass accuracy are exemplified. m/z_{meas} and m/z_{theor} stand for measured and theoretical m/z (adapted from [6]).

varies between 20,000 and 140,000 in (U)HPLC-MS analysis for *m/z* between 100 and 800. Orbi-MS has a higher R allowing a better discrimination of ions (e.g., fine isotopic distribution).

This is also of interest to know that there are slow and fast-scanning MS. In scan mode (e.g., *m/z* ranging from 100 to 1000), QQQ-MS are slow and insensitive. For this reason, the acquisition mode of QQQ-MS is, in most cases, selected reaction monitoring (SRM; also called multiple reaction monitoring, MRM). In SRM acquisition, the precursor ion is selected in the first quadrupole (Q1) and fragmented in the collision cell (Q2), and an intense and specific fragment/product ion is selected

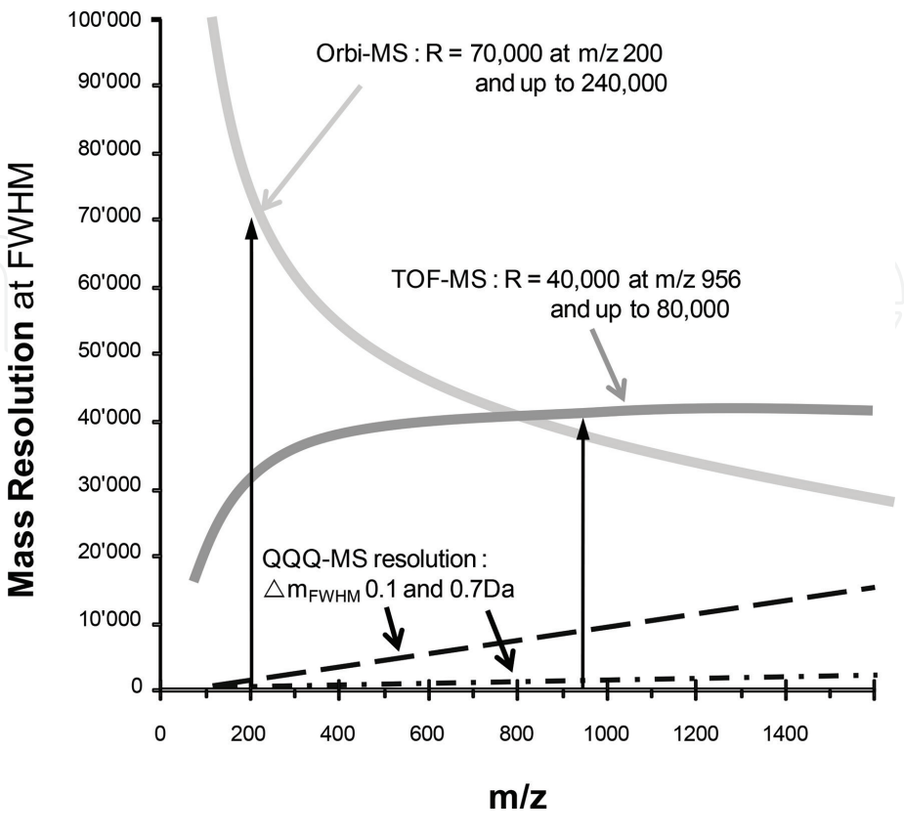

Figure 8.
Mass resolution (R) against the m/z range for the three most frequent MS technologies (adapted from [6]).

in the last quadrupole (Q3) prior its detection by the photomultiplier. This is an ion transition please, see **Figures 6** and **9**. In contrast, TOF- and Orbi-MS are fast-scanning instruments. Scan rates of affordable Orbi-MS depend on the resolution chosen and are typically 1.5, 3, 6, and 12 Hz (number of scans/s) at R = 140,000, 70,000, 35,000, and 17,500 (at *m/z* = 200), respectively. TOF has a higher scan rate (>20 Hz) which can be an advantage if multiple acquisitions are recorded in parallel or if ion mobility is used. The main differences between the three most frequent technologies, QQQ-, Orbi-, and TOF-MS, are depicted in **Figure 6** and **Table 1**.

Figure 10 sketches the key consequence of the differences between QQQ-MS, on one side, and TOF- and Orbi-MS, on the other side. This is related to the acquisition types that are ion transitions (SRM) and high-resolution full scan (HR-full scan). QQQ-MS *have to* acquire SRM in order to be selective and sensitive, whereas TOF- and Orbi-MS are selective *and* sensitive in the full-scan mode acquisition [13]. This means that with SRM acquisition, the bioanalyst has to know the precursor and product ions and the collision energies to apply *prior* to the analytical run. In contrast, with HRMS full-scan acquisition, the bioanalyst can use generic parameters and acquire all ions in a large mass range (e.g., *m/z* from 100 to 1000) **Figure 9**. In this last scenario, a global determination is recorded allowing various kinds of retrospective, targeted, or untargeted data treatments. In this book chapter, we have defined the global ion acquisition as the HR-full scan, whereas there are various alternative possibilities defined as data-independent acquisitions (DIA; e.g., MS^{ALL}, MS^{E}, MS^{SWATH}) that also record virtually all ions [14, 15].

A good example of retrospective data mining is shown in the biotransformation study of tamoxifen [16]. In this study, plasma samples from patients treated with tamoxifen were extracted and analyzed with a LC-HRMS acquiring HR-full scan. Retrospective data treatment allowed to detect and determine 39 tamoxifen metabolites. A second example of post-acquisition data mining is shown in an

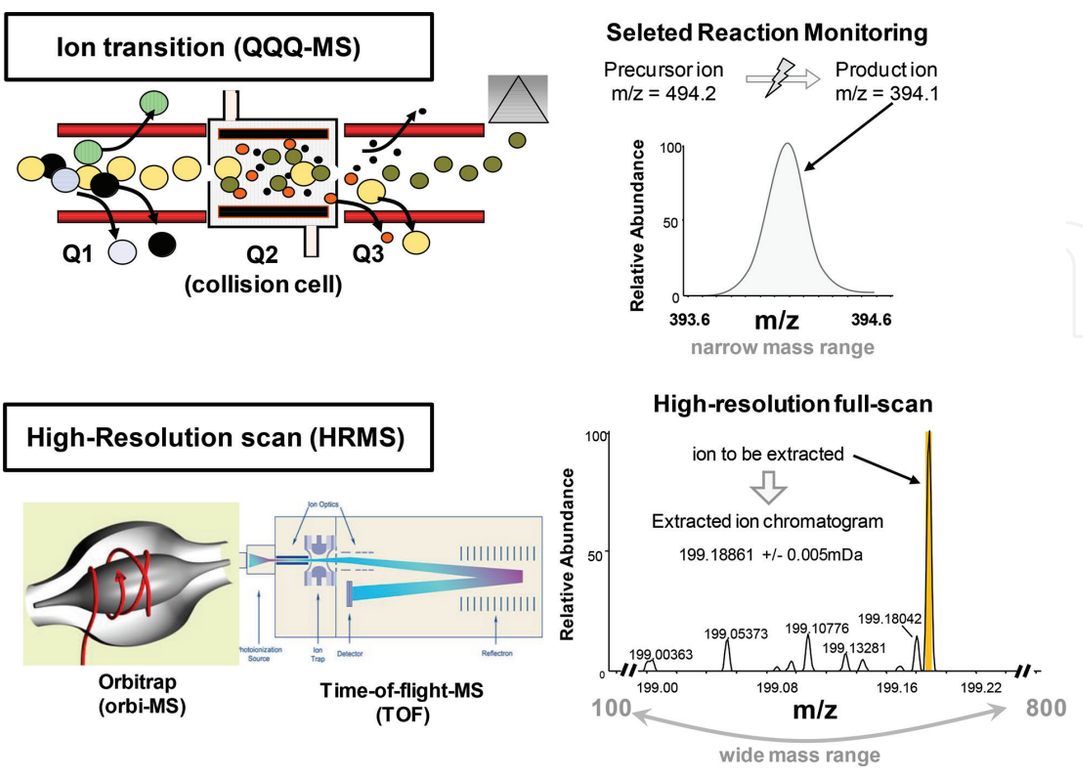

Figure 9.
Typical acquisitions for sensitive and selective detections with low- and high-resolution MS: ion transitions (QQQ-MS) and full scan (HRMS), respectively.

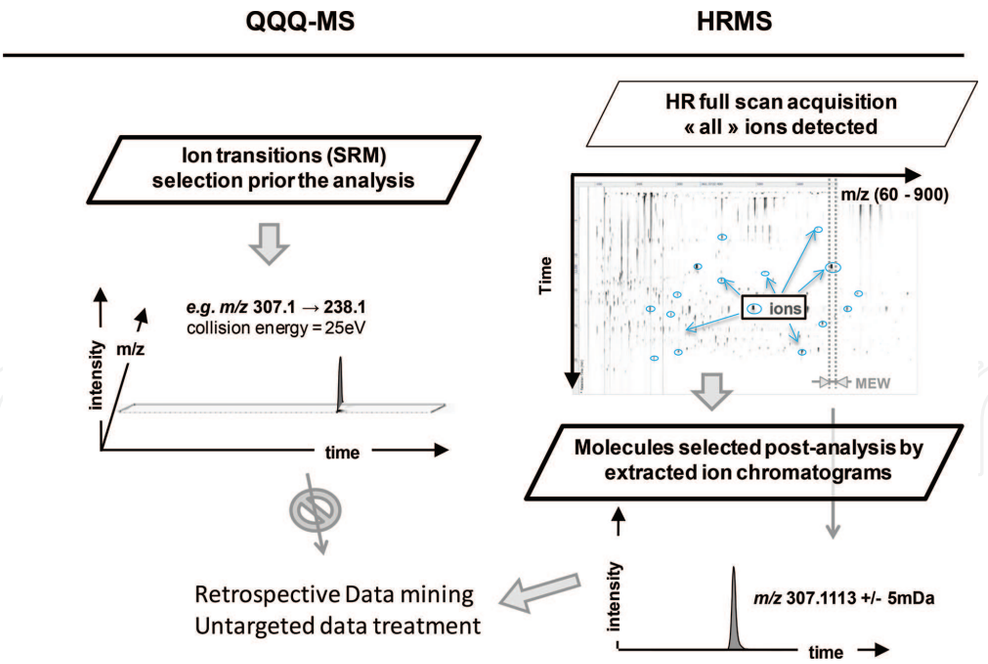

Figure 10.
Usual acquisitions for sensitive and selective detections on QQQ-MS and HRMS analyses. With QQQ-MS, ion transitions have to be defined before the analysis. Therefore, the selective filter is applied before the acquisition. With HRMS, the acquisition can be performed in full-scan mode where, virtually, "all" ions are recorded. The selective filter is applied after the acquisition by choosing the ions to be extracted. Typical QQQ- and HRMS acquisitions are depicted on the chromatograms.

untargeted diagnostic screening workflow where an outlier metabolite in a patient's metabolome is revealed by the comparison with healthy people metabolomes [17]. Both examples are better described in Section 3.

Importantly, like for QQQ-MS, most actual HRMS can perform excellent quantitative determinations (see the following section) [13]. This is why today's HRMS instruments should be considered as the new gold standard in LC-MS analyses because of their capabilities to perform quantitative, qualitative, quan/qual, and omics analyses (**Figure 11**) [13]. As depicted in **Table 1**, quantitative performance of HRMS is not better but equivalent to QQQ-MS. But the

		QQQ-MS	Q-TOF-MS	Orbi-MS
	Mass Resolution	low	high	high
Sensitivity	Global analysis *	1	3	3
	Targeted analysis **	3 (4)	(2) 3	(2) 3
Specificity	Global analysis *	1	(2) 3	3 (4)
	Targeted analysis **	3	(3) 4	4 (5)
Acquisition rate	Global analysis *	1	(3) 4	3
	Targeted analysis **	3	(2) 3	2
Sum	Global analysis *	3	**8-10**	**9-10**
	Targeted analysis **	9-10	**7-10**	**8-10**

Table 1.
Key analytical differences of the most frequently used mass spectrometers. This table allocates a grade according to global or targeted MS performance (one to five points for poor to excellent, respectively). Global acquisition (*) corresponds to high-resolution full scan or data-independent acquisition (no selection of precursor ions). Targeted acquisition (**) corresponds to SRM (selected reaction monitoring) or product ion scan (selection of precursor ions). The last row gives the sum of the grades (adapted from [7]).

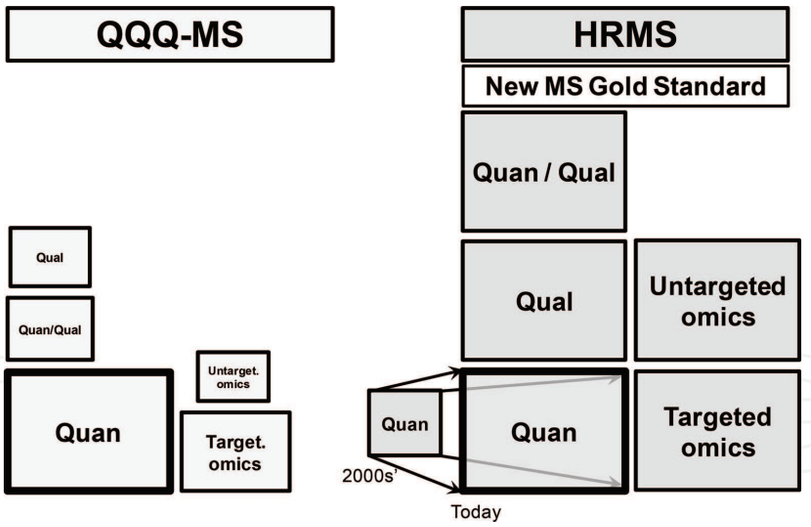

Figure 11.
Overview of the analytical workflows performed by QQQ-MS and HRMS. Box sizes relate to the analytical performance. Whereas quantitative (quan) determination is similar between LRMS (SRM; QQQ-MS) and HRMS (TOF-, Orbi-MS; HR-full scan), HRMS has much better analytical capabilities for qualitative (qual), simultaneous quantitative and qualitative (quan/qual), and omics (targeted and untargeted metabolomics and proteomics) workflows.

HRMS-unique capability to perform global and qualitative analyses is becoming more and more crucial. For one or two decades, the mind-set of bioanalysts performing LC-MS analysis was the following: QQQ-MS are the instruments of choice for routine and quantitative analyses, whereas complex and expensive HRMS instruments are dedicated to qualitative and research analyses. This is outdated. With the recent progress in HRMS technologies (both TOF and Orbi-MS), a shift of paradigm is occurring [8, 12, 18]. This shift of paradigm is based on the fact that today's HRMS instruments can perform *both* excellent quantitative and qualitative analyses. HRMS should be seen as the new gold standard mass analyzers (**Figure 11**).

In the next section, typical examples of LC-HRMS analyses are presented. HRMS represents the most versatile analytical tool allowing to perform from targeted quantification to untargeted metabolomics/proteomics [13].

3. Examples of quantitative and qualitative LC-HRMS analyses

3.1 Quantitative performance of HRMS instruments

Today, there are many peer-review articles that compare side-by-side the quantitative performance of QQQ-MS and HRMS instruments [19–26]. These comparisons investigate the sensibility, selectivity, ease of use, calibration linearity, reliability, and robustness in real-life LC-MS analyses. This is always difficult to have an absolute comparison between two MS technologies because it is related to various parameters like the compounds studied, the two ion source designs (not the MS itself) that possibly show different sensitivities, the memory effects on column that perturb the determination of the limit of detection, the different generations of lenses/ion guides that are just behind the entrance of the MS and that usually are independent to MS technologies, etc. The frequently claimed better sensitivity of QQQ-MS over other technologies is often the result of latest hardware modifications (more efficient lenses, ion guides, etc.) introduced first in new QQQ-MS. Again, this is not related to the mass analyzer itself.

Nevertheless, a general conclusion can be drawn from these numerous articles and comparisons. Today, most HRMS instruments show similar quantitative performance than QQQ-MS [13, 19–26]. In **Figures 12–14**, adapted from recent publications (respectively Refs. [25] and [23]), the authors underscore that HRMS quantitative performance is comparable to QQQ-MS. **Figure 12** depicts LC-MS chromatograms at the limit of detection, the calibration curves, and the fold differences of the limits of detection obtained for nine drugs with a QQQ-MS (TSQ Quantum Ultra) performing ion transitions (SRM) and a HRMS (Q Exactive Focus) performing HR-full scans. No significant differences of quantitative performance were observed except the saturation of the QQQ-MS detector at the highest spiked concentrations (5000 ng/mL corresponding to 10 ng on column) [25]. **Figure 13** depicts the limits of quantification (LOQ) of 27 compounds determined in SRM and full scan with a QQQ-MS and a HRMS [23]. Here again, no significant differences in the LOQ values were observed [23]. As shown in **Figure 13**, the fold differences between QQQ-MS and Orbi-MS are below ≈3x (or 1/3x) for 22/27 compounds. Four compounds show a more pronounced difference, between 7x and 20x, in favor of HRMS, which is probably the result of poor intensity of the fragment ions (SRM acquisition). In contrast, one compound shows a better LOQ value (8x) with the QQQ-MS. **Figure 14** shows the reliability of 25-hydroxyvitamin D3 quantification in 662 serum extracts analyzed with a QQQ-MS (SRM) and an Orbi-MS (HR-full scan) [27], exemplifying the robustness of HRMS in routine and quantitative determinations (see also [28, 29]). Similar conclusions showing the

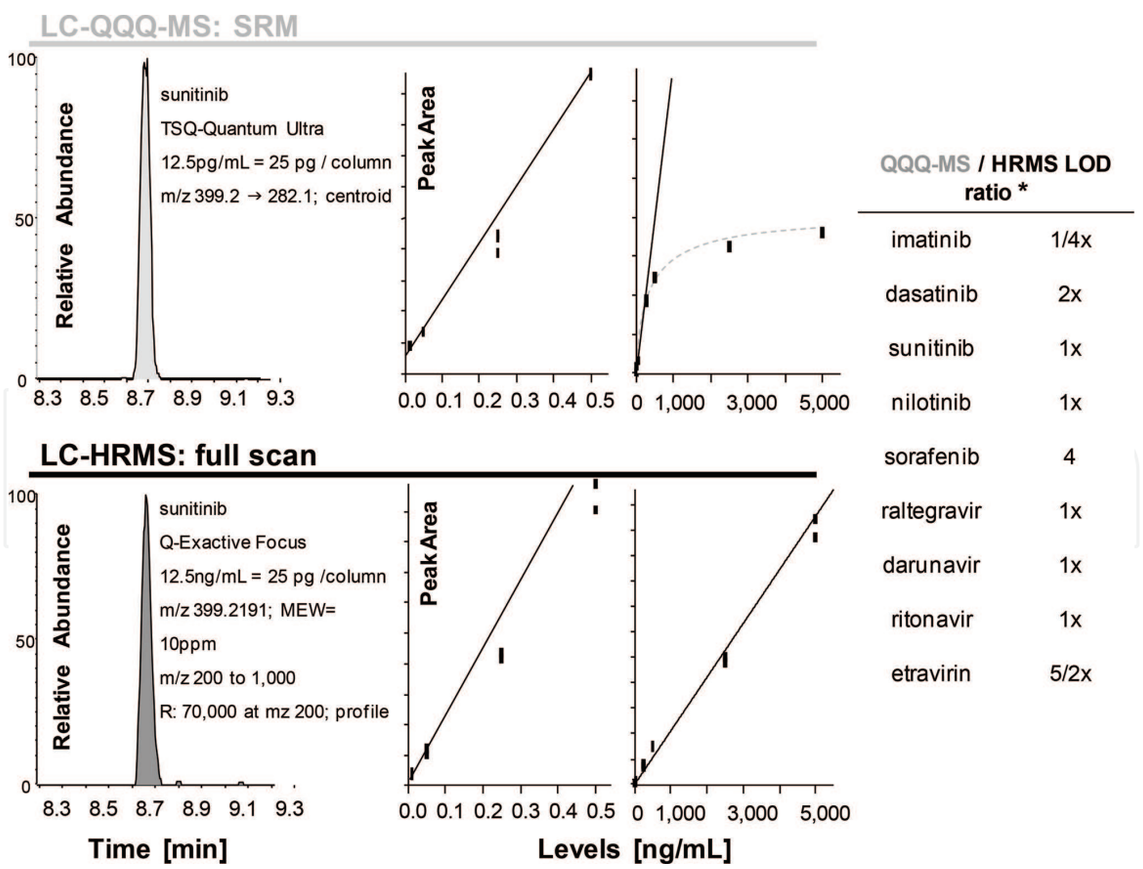

Figure 12.
Example of side-by-side comparison of LC-MS quantification with QQQ-MS and HRMS. Chromatograms at the limit of detection (LOD) in plasma extracts, curve linearities, and LOD differences between both MS platforms are depicted, whereas QQQ-MS and HRMS acquire SRM and HR-full scan, respectively. Calibration curves at the lowest levels are similar, whereas at the highest levels, a saturation of the QQQ-MS multiplier is observed. (*) ratio < 1 means QQQ-MS is more sensitive than HRMS and vice versa (adapted from [14]).

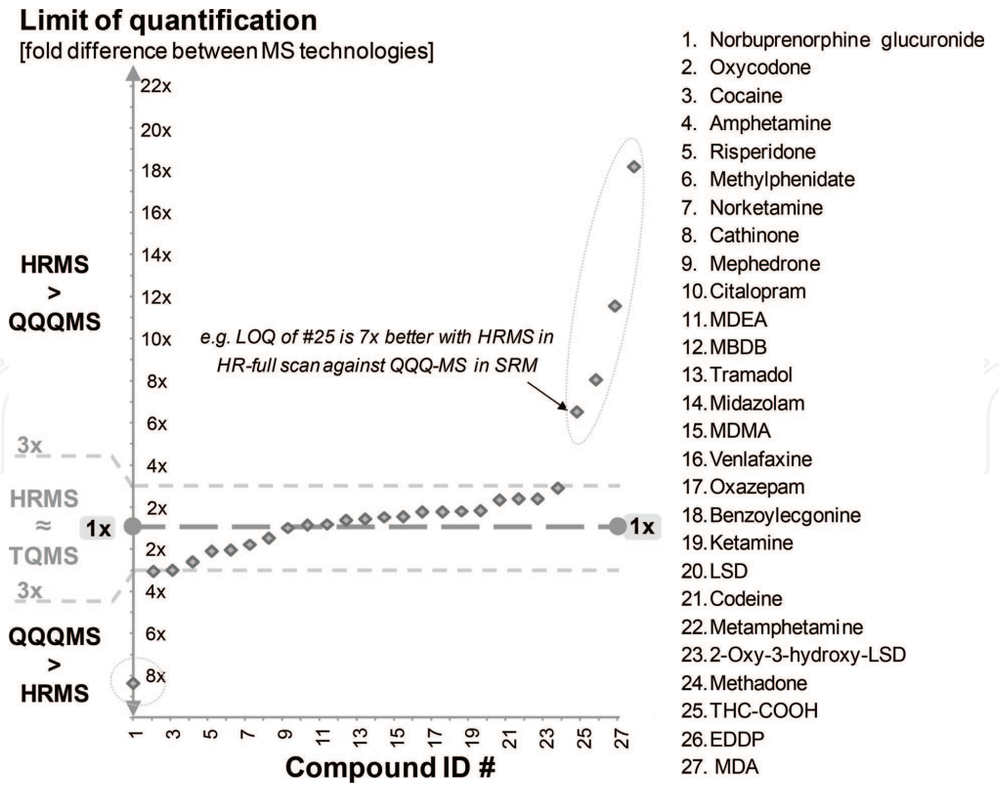

Figure 13.
Example of side-by-side comparison of LC-MS quantification with QQQ-MS and HRMS. Limits of quantification for 27 compounds are plotted in biological extracts. Most compounds show similar LOQ values (differences <3x), whereas four compounds with probable poor intensity of the fragment ions show lower LOQ values with QQQ-MS (adapted from [20]).

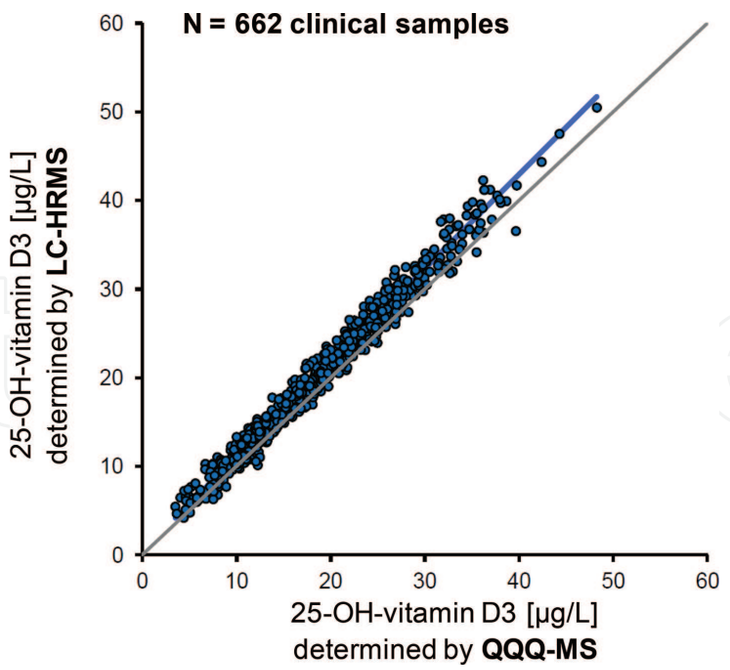

Figure 14.
Passing and Bablok fit of 25-hydroxyvitamin D3 in 662 plasma extracts determined with QQQ-MS and HRMS acquiring SRM and full scan (adapted from [16]).

capability of routine determinations have been made for Q-TOF-MS [20, 30, 31]. If needed (e.g., poorly cleaned sample extracts or direct-flow-injection analysis), the acquisition of fragment ions can show higher selectivity or/and sensitivity in LC-HRMS analysis [25, 32].

Evidences of HRMS quantitative performance are accumulating [13], but this is not straightforward to change bioanalysts' habits that have used QQQ-MS for decades. It can be difficult to convince them to shift, whereas HRMS shows "only" similar quantitative performance as QQQ-MS. The shift in favor of HRMS still needs activation energy in order to occur. This activation energy will come from the additional and outstanding capabilities of HRMS for qualitative and untargeted determinations and the increasing needs for more global and/or untargeted approaches in the determination of biological samples [33]. Even if some laboratories will concentrate their work exclusively on targeted and quantitative analyses and will possibly keep working with predefined ion transitions on QQQ-MS, most lab heads will "feel" the additional needs for untargeted acquisitions and untargeted data treatments for routine or investigation analyses (see Introduction section and the following section). Here below, quan/qual, qualitative, and omics analysis are presented.

3.2 Quan/qual and qualitative analyses with HRMS instruments

The in vivo biotransformation study of tamoxifen represents a good example of quan/qual analysis [16]. Tamoxifen is a selective estrogen receptor modulator, used for the prevention and adjuvant treatment of estrogen-sensitive breast cancer. In this study, plasma samples from patients treated with tamoxifen (steady-state levels) were analyzed with a QQQ-MS (TSQ Quantum Ultra; SRM acquisition) or with an Orbi-MS (Exactive Plus MS; HR-full-scan acquisition). It has been shown that tamoxifen metabolites can be more active than the parent drug and that tamoxifen reactive metabolites can bind to macromolecules [33]. It was and is challenging to reveal new findings with tamoxifen because hundreds of articles have already studied *in vivo* and *in vitro* the biotransformations of tamoxifen [21].

First, in this study [16], similar plasma levels (CV < 15%) of tamoxifen and two metabolites were obtained with the QQQ-MS and the HRMS platforms. Secondly, the HR-full-scan acquisition allowed the identification of 39 circulating metabolites of tamoxifen, of which 3 were never described previously and corresponded to the sixth and seventh generations (6 and 7 biotransformation steps; **Figure 15**). Semiquantitative determinations of tamoxifen metabolites allowed to observe that some metabolites have a high plasma level variability with poor relations with the parent drug level, whereas, in contrast, other metabolites show a strong relation to tamoxifen levels (**Figure 15**). Various strategies were employed to identify tamoxifen metabolites. They consisted in the extraction of ions from possible biotransformation pathways (and chemical compositions) or from similar tamoxifen fragmention (**Figure 15**). All these tasks were particularly simplified because of the HRMS detection selectivity (accurate mass and high-resolution spectra allowing narrow mass-extraction-windows) and because of the sensitivity of HR-full scan or MS^{ALL} acquisitions (product scan where all precursor ions are fragmented, no selection of precursor ions in the quadrupole; see also data-independent acquisition, MS^{SWATH}, or MS^{E} acquisitions [13]).

This study [16] demonstrates the excellent capabilities of HRMS data for further investigations in real biological samples on top of the targeted and quantitative determinations of tamoxifen and two metabolites. Similarly, from HR-full-scan acquisition, new metabolites of hepcidin, a peptide involved in iron homeostasis in the blood, have been discovered on the side of the quantitative determination of hepcidin [28]. It underscores that HR-full-scan acquisitions allow targeted simultaneous and quantitative determinations and investigational data treatment (see also [14, 34–37]).

3.3 Omics analyses with HRMS instruments

In general, LC-MS omics analyses (metabolomics and proteomics) are associated to research analyses such as the discovery of biomarkers. In the new understanding

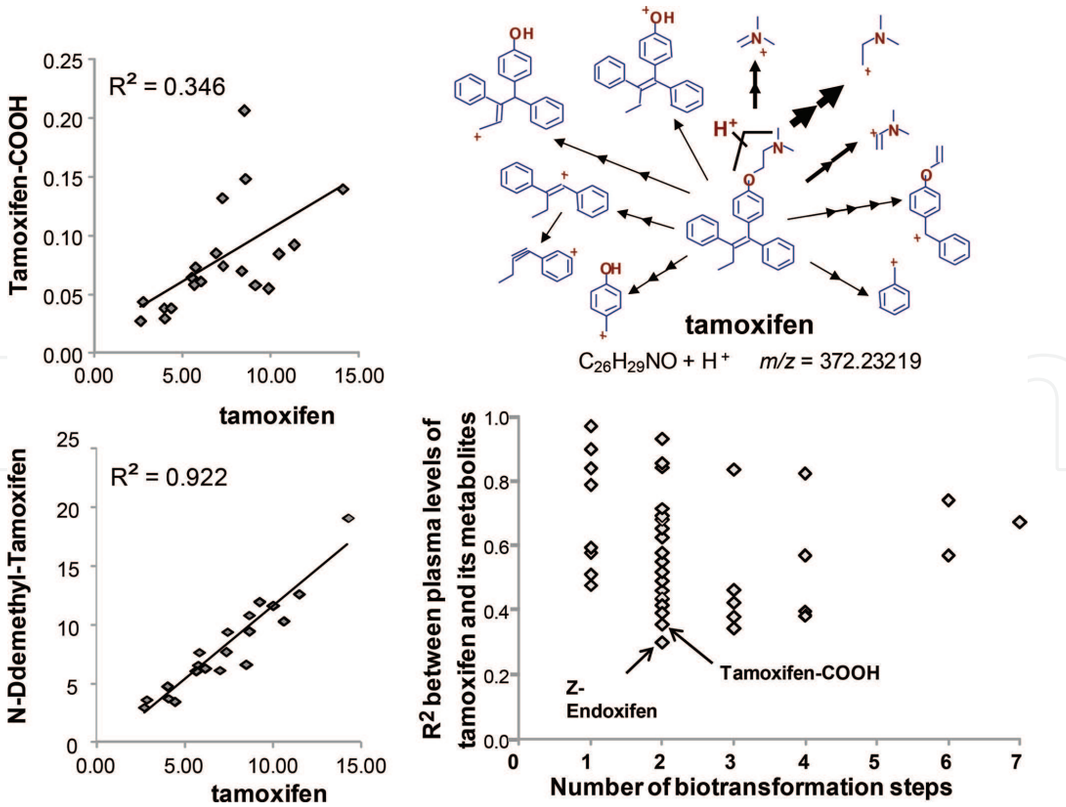

Figure 15.
Biotransformation study of tamoxifen. Left-hand side: correlations between tamoxifen levels and two of its metabolites showing poor (top) and good (bottom) R^2 coefficient. Right-hand side: top, tamoxifen fragmentation; accurate mass determination allow to assign chemical compositions and putative chemical structures to tamoxifen fragments; bottom: in-depth coverage of tamoxifen metabolite discovery resulting from HR scans and accurate mass extractions of ions (adapted from [9]).

of life sciences where a holistic approach has its place in routine analysis and where low-prevalence diseases represent a significant part (**Figure 4**), there is an increasing need for untargeted or semi-targeted LC-MS analysis as a diagnostic workflow. Here below, an untargeted diagnostic screening is presented as a metabolomics analysis [17]. This study evaluates the feasibility to perform an untargeted LC-HRMS analysis and an untargeted data treatment in a routine context.

Figure 16 depicts this study [17] and its omics workflow. *One* serum sample (the test sample) was spiked with *one* compound. The serum sample was then extracted (protein precipitation) and analyzed with a LC-HRMS system acquiring HR-full scan from *m/z* 60 to 900 (untargeted acquisition) this sample was compared with 95 samples from healthy persons. Unsupervised (untargeted) data treatment with a metabolomics software allowed, after the application of different filters that remove insignificant ions (>99%), to reveal the spiked compound in a short and final list of 20–50 compounds (the number of detected ions is >10,000). Whereas, in the final list, many ions were not identified (e.g., unknown adducts, isotopes, or in-source fragments) or were compounds from food intake; this untargeted diagnostic screening procedure appears feasible and reliable to reveal unexpected xenobiotics (toxicology) or higher concentrations of endogenous metabolites. Correlation(s) between clinical symptoms and metabolite outlier(s) found in a patient should be done by clinicians prior to the request for a confirmatory, targeted, and absolute quantification analysis. Such HRMS untargeted approaches could be useful as preliminary diagnostic screening when canonical and targeted processes do not establish nor reveal a clear diagnostics or disease etiology (low-prevalence disease mechanism or etiology). Similar examples that underscore or suggest the utility of untargeted acquisitions in routine analyses have been published [38, 39].

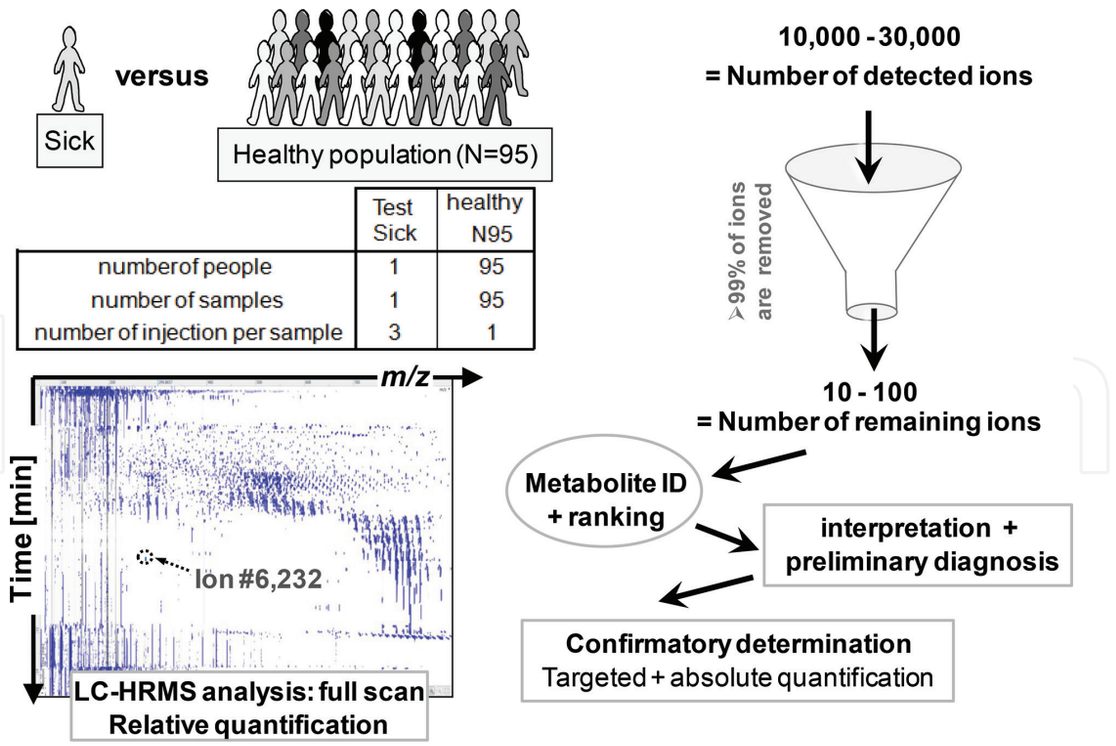

Figure 16.
Untargeted diagnostics screening workflow. The metabolome of a human serum (containing a spiked molecule, "sick") is determined and compared with 95 healthy serum metabolomes. HR-full scans are acquired and untargeted data treatment is performed with a metabolomics software. After removing useless ions with proper filters, the spiked molecule was identified and ranked. Revealed metabolites can be related to the patient's symptoms, preliminary diagnosis postulated, and further confirmatory analysis performed. Such untargeted analysis can be undertaken when canonical procedures fail. This workflow is in accordance with "low-prevalence diseases" and against the "tyranny of the average" concepts (adapted from [8]).

4. Conclusion and perspectives

In this book chapter, a brief review of the performance of HRMS instruments has been presented: first, on quantitative and then on qualitative, quan/qual, and omics analyses. Today, there are numerous peer-review articles showing that in quantitative analysis, most actual HRMS instruments are performing similarly to QQQ-MS. Taken into account the additional and unique HRMS capabilities for qualitative and untargeted determinations, HRMS opens new analytical possibilities that fit new requests from systems biology and its holistic/global approaches. For this reason, HRMS should be considered as the new gold standard MS systems (paradigm shift). Indeed, HRMS shows unique versatility, and bioanalysts can foresee routine or research analyses from targeted quantifications to untargeted metabolomics.

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Conflict of interest

The author declares no conflict of interest.

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Author details

Bertrand Rochat
Département Formation Recherche, University Hospital of Lausanne,
PAF, Génopode, Université de Lausanne, Lausanne, Switzerland

*Address all correspondence to: bertrand.rochat@chuv.ch

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