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LC/MS: AN ESSENTIAL TOOL IN DRUG DEVELOPMENT

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ABSTRACT

The combination of high-performance liquid chromatography and mass spectrometry (LC/MS) has had a sign cant impact on drug development over the past decade. Continual improvements in LC/MS interface technologies combined with powerful features for structure analysis, qualitative and quantitative, have resulted in a widened scope of application. These improvements coincided with breakthroughs in combinatorial chemistry, molecular biology, and an overall industry trend of accelerated development. The use of high-performance liquid chromatography combined with mass spectrometry (HPLC–MS) or tandem mass spectrometry (HPLC–MS–MS) has proven to be the analytical technique of choice for most assays used in various stages of new drug discovery. A summary of the key components of HPLC–MS systems, as well as an overview of major application areas that use this technique as part of the drug discovery process, will be described here. This review will also provide an introduction into the various types of mass spectrometers that can be selected for the multiple tasks that can be performed using LC–MS as the analytical tool. The strategies for optimizing the use of this technique and also the potential problems and how to avoid them will be highlighted**.**

***Keywords*:** LC-MS analyticaltool, HPLC-MS, HPLC-MS-MS, LC-MS

1. Introduction:

Current trends in drug development emphasize high volume approaches to accelerate lead candidate generation and evaluation. Drug discovery-based technologies that involve bimolecular screening and combinatorial chemistry paved the way, resulting in shortened timelines and the generation of more information for more drug candidates. The impact on the overall drug development cycle has been sign cant, creating unprecedented opportunities for growth and focus, particularly in the analytical sciences The use of high-performance liquid chromatography combined with mass spectrometry (HPLC–MS) or tandem mass spectrometry (HPLC–MS–MS) has proven to be the analytical technique of choice for most assays used in various stages of new drug discovery [1].New drug discovery can be defined as the process whereby compound libraries are screened, then hits are selected and modified to become leads that are optimized until a compound emerges that can be developed into a drug candidate. HPLC–MS and HPLC–MS–MS are used for the analysis of newly synthesized compounds that become part of a compound library. These assays check that the correct compound has been synthesized and that the purity is sufficient to be used in the library. In a second stage, various physical and chemical properties (e.g. physiological solubility, permeability and chemical stability) of these new chemical entities (NCEs) are assessed and HPLC–MS is often used for these assays

2. Principles of LC–MS: The elements of an LC–MS system include the auto sampler, the HPLC system, the ionization source (which interfaces the LC to the MS) and the mass spectrometer. Ideally, these elements are all under the control of a single computer system. HPLC is a common technique, so it will not be described here. It should be noted that to interface HPLC with MS, there are some restrictions on the flow rate and mobile phases that can be used .Typical reversed phase HPLC systems connected to MS would use some combination of water and either methanol or acetonitrile as the mobile phase. There are limitations on the mobile phase modifiers; for example, in most cases the modifiers have to be volatile. Mobile phase modifiers are chemicals added to the mobile phase that are used primarily to improve the chromatography of the Ana lutes of interest. Typical mobile phase modifiers would include ammonium acetate, acetic acid and formic acid. There are multiple articles that focus on the HPLC parameters that are important in LC–MS assays.

3. Types of mass spectrometers:

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There are many types of mass spectrometers available for interfacing with HPLC[2] , one of the more common systems used for HPLC–MS is the single quadruple mass spectrometer; this system will provide a mass spectrum for each chromatographic peak that elutes from the LC column and is analysed by the MS system. The second type of system shown is the time-off light (TOF) mass spectrometer, which has the added capability of providing a higher mass resolution spectrum from each component that is assayed. The third system shown is the triple quadruple MS–MS system, which is most often used for bio analytical assays but can also be used for metabolite identification assays [3]. The fourth MS system is called an ion-trap mass spectrometer and has the unique capability of producing MS data that are important when performing structural elucidation assays. In addition to these four types of mass spectrometers, there are a growing number of additional types, including hybrid systems that have unique capabilities. Hybrid mass spectrometers combine two of the basic types of mass spectrometer to make a specialty system; an example of a hybrid mass spectrometer is the 'Q-TOF' MS–MS system, which combines a quadruple mass spectrometer with a TOF mass spectrometer.

4. Integration of LC/MS into Drug Development

Liquid chromatography/mass spectrometry (LC/MS)-based techniques provide unique capabilities for pharmaceutical analysis. LC/MS methods are applicable to a wide range of compounds of pharmaceutical interest, and they feature powerful analytical gores of merit (sensitivity, selectivity, speed of analysis, and cost effectiveness). These analytical features have continually improved, resulting in easier to use and more reliable instruments. These improvements were timely and coincided with the aforementioned developments in the pharmaceutical industry.

4.1. Emerging Analytical Needs: Perhaps a major cause of these opportunities is the fact that the rate of sample generation far exceeded the rate of sample analysis. To put this factor in perspective, consider the following example that deals with combinatorial chemistry. Prior to the advent of combinatorial chemistry technologies, a single bench chemist was capable of synthesizing ca. 50 ®nil compounds per year, depending on the synthesis. Today, chemists are capable of generating well over 2,000 compounds per year, using a variety of automated synthesis technologies. If traditional approaches to analytical support were maintained, then analysts would outnumber chemists by nearly 40 to 1!

* An earlier availability of information leads to faster decision making.

* Integration of instrumentation with information network is a popular approach for combining high throughput analytical information generation with Drug-candidate screening.

* Software is a powerful resource for the coordination of analysis events and the management and visualization of data.

4.2. Partnerships and Acceptance: What has happened in the pharmaceutical industry during this relatively short time span is truly remarkable. With the advent of advanced technologies responsible for increasing the rate of sample generation, there is strong motivation to respond with LC/MS-based analysis Techniques. The understanding of principles, fundamentals, operation, and maintenance enabled researchers to improve analytical performance. Here, the power of `seeing is believing" led to lower barriers of acceptance as well as to a new breed of practitioners

5. Systematic LC/MS Metabolite Identification in Drug Discover:

The study of how a drug is absorbed, distributed, metabolized, and eliminated by the body is a vital but costly and time-consuming step in the drug discovery process. Metabolism can dictate the rate of absorption into the body, lead to the production of new and possibly toxic species, or activate the drug. For example, morphine's effect is primarily due to one of its glucuronide conjugate metabolites .Until recently, metabolite identification only took place once a compound had been chosen for drug development. However, the discovery of a toxic metabolite can set a research program back significantly. As a result, many pharmaceutical companies are now conducting metabolite identification studies in the early phases of drug candidate selection. But this is no easy task. The metabolism of a drug within a test animal can be extremely complex, involve multiple enzymatic pathways, and lead to a range of compounds with varying concentrations ([7]). Other drugs have one or two major metabolic pathways that dominate their metabolism, but several minor pathways can

produce at least one metabolite. We have seen >30 detectable, and theoretically identifiable, metabolites form from a single compound.

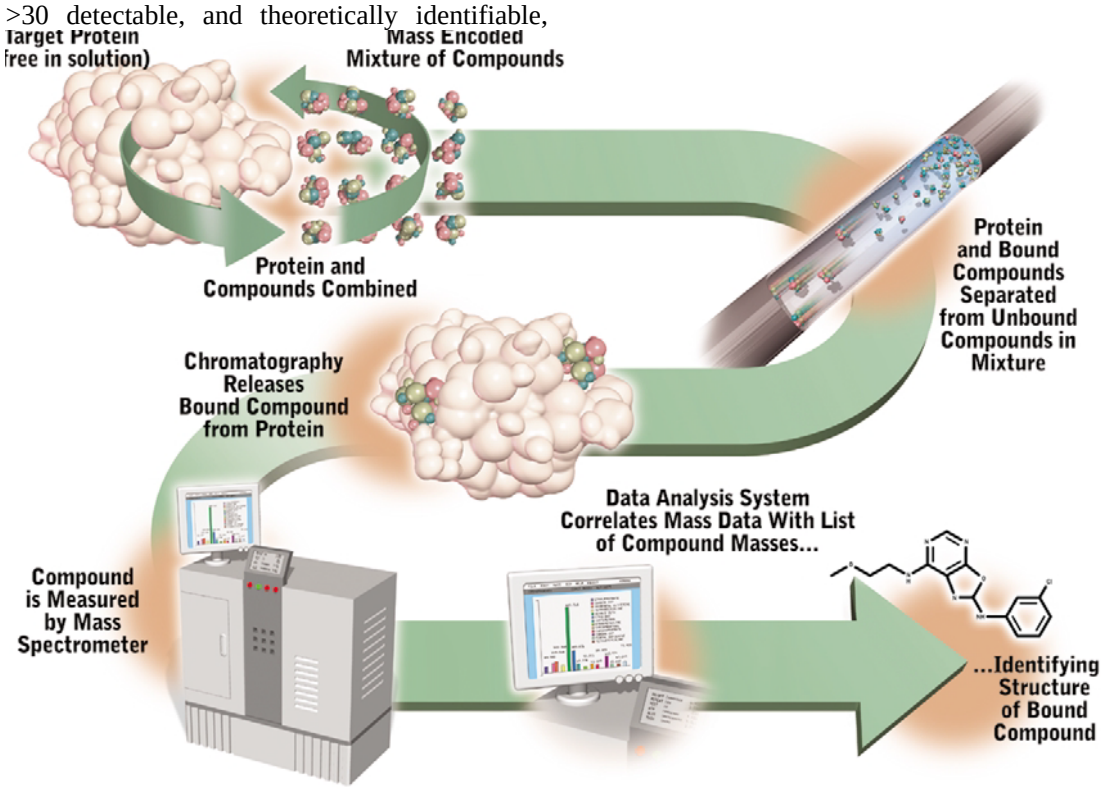


Figure1: Diagram showing how the Automated Legend Identification System (ALIS) system uses MS for high throughput activity screening.

5.1. Chemistry-library synthesis: In a typical arrangement, an HPLC–MS system will be used to provide information on compound identity and purity as a first step in building a discrete compound library. A common system would have an in-line UV detector for the purity assessment, as well as the MS system for the compound identity confirmation. The mass spectrometer is often either a single quadruple or a TOF system. These systems are often highly automated; for example, Isbell *et al*. [24] describe a high throughput procedure that combines an automated compound purification procedure with the compound analysis step. This process is based on a combination of HPLC–MS with software-controlled fraction collectors that are triggered on the basis of the observed or expected *m/z* response of the compound of interest to make the whole process highly automated.

5.2. *In vitro* screening: One of the more commonly used *in vitro* screens is the human colon adrenal carcinoma cell line (Caco-2), which is used for the measurement of the permeability potential of a compound – one of the aspects of the absorption process [31].There are several reports on how LC–MScan be used for the analysis of Caco-2samples [34]. In one example, Fung *et al.*[35] described a higher throughput assay for Caco-2 samples that was capable of handling 100 compounds per week, based on HPLC–MS–MS using a triple quadruple MS system. One of the tools required to assay this many compounds was an MS method development tool, provided as part of the software package by the instrument vendor; this tool is important for applications that require the system operator to develop discrete MS–MS transitions for each compound that is assayed. Another way in which Fang*ed al*. [32] improved the assay efficiency was by reducing the number of samples that had to be injected through the elimination of a calibration curve. The Caco-2 results for a given compound (permeability calculation) are based on the ratio of two samples; therefore, they demonstrated that the ratio of the MS responses of the two samples could be used instead of the ratio of the concentrations of the two samples, thereby eliminating the need for a calibration curve for each compound. Another higher throughput *in vitro* assay is the one used to assess a potential of a compound to inhibit of one or more of the human cytochrome P450 isoforms (CYPs); this

step is important to determine a compound's potential for drug–drug interactions. In this case, the assay can be optimized to be high throughput because the analysis does not measure the compound that is being tested. There have been several reports in recent years describing how HPLC–MS–MS can be used for providing higher throughput assays to support various CYP screens for enzyme inhibition .In a recent example, Pang *et al*. described a high throughput assay based on HPLC–MS–MS to screen for five important CYP ribozymes – CYP 3A4, CYP2D6, CYP2C9,CYP2C19 and CYP1A2.

5.3. High-throughput screening: Although HTS is usually performed using various fluorescence procedures to look for compounds that have the desired *in vitro* activity, there have been some examples where mass spectrometry has been used for this step in the new drug discovery process As discussed by Flab and Jindal [26], the strategy uses mixtures of compounds plus a target protein to identify potential lead compounds for a therapy based on compound so that interact with the target protein. This high throughput screen uses HPLC–MS to identify the ligand compounds; in this case the mass spectrometry system is a TOF.

5.4. ADME–PK screening: Perhaps the most common use for LC–MS in new drug discovery is for the various ADME studies that make up the majority of the effort provided by the drug metabolism and pharmacokinetic (DMPK) groups in their participation in the process [27–30]. There are several *in vitro* ADME screens, followed by various *in vivo* preclinical ADME–PK screens. These screens are almost always supported by LC–MS assays.

6. *In vitro* screen is the metabolic stability assay:

The goal of this assay is to provide a prediction of the *in vivo* intrinsic clearance of a compound. Aniseed and Thacker recently reviewed the area of metabolic stability assays and also provided a good summary of the relative importance of the major CYP isoforms for human metabolism. Generally, the metabolic stability assays are based on the incubation of the compound in the presence of either human liver microtomes or human hepatocytes; in either case, the samples are typically assayed using a compound-specific analysis based on HPLC–MS–MS (usually with a triple quadruple system). Several examples of high throughput metabolic stability assays based on LC–MS have been reported. In an early example, Korfmacher *et al*. [44] described an automated assay based on a single quadruple LC–MS system that used an automated data analysis system and could test 75 compounds per week for metabolic stability. Wring *et al*. [36] described a system for metabolic stability that included automated liquid handling and an LC–MS assay based on a triple quadruple mass spectrometer that was capable of handling 50 compounds per week. More recently, Xu*et al*. [30] reported a highly automated system based on robotic sample preparation of the test compound plates, as well as the human liver microsomal incubations with three time points selected (5,15and 30 min) in addition to the time zero point. All samples were measured in triplicate to improve the reliability of the results. The assay was based on a single quadruple LC–MS system that included an eight-probe auto sampler and eight HPLC columns in a parallel mode. This system was able to assay 240 samples per hour, which enabled up to 176 test compounds to be evaluated per day. In addition to the high throughput assay, the authors described automated data processing tools that enabled the analysis of this data in a short time frame.

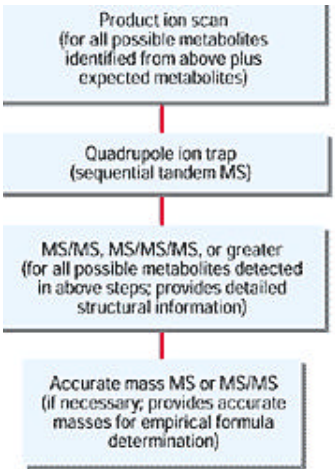
7. LC/MS Metabolite Identification in Drug Discovery:

MS has emerged as an ideal technique for the identification of such structurally diverse metabolites. When coupled with on-line HPLC, the technique is extremely robust, rapid, sensitive, and easily automated [11]. Not surprisingly, LC/MS and LC/MS/MS have become the methods of choice for pharmacokinetic studies, yielding concentration versus time data for drug compounds from in vivo samples such as plasma [2].Instruments and software packages. Furthermore, just as in a police investigation Nevertheless, identifying metabolites remains a time-consuming process because arrange of instrumental techniques and software applications are needed to obtain the appropriate data. The analyst must be quite experienced to handle these various the data is rarely obvious or completely conclusive, but rather requires previous experience to unravel it. For all these reasons, interpreting the data is typically the largest bottleneck in metabolite identification.

In this report, we present the choices and decisions involved in metabolite identification and how they can be merged into a systematic approach. The discussion covers the tools and techniques available to the mass spectra merits, the complementary natures of different types of

mass spectrometers, and innovative software that reduces the size of data sets. This new approach greatly increases sample throughput and turnaround time for useful information. A complete metabolite identification study is also described, which uses all of the techniques and instrumentation discussed.

The flow chart in the basic approach to metabolite identification:



8. Systematic approach to metabolite identification and characterization:

The approach assumes that it is possible to predict numerous common alterations to the drug such as oxidation and oxidative conjugation. Typically, the compounds under investigation in a single drug discovery project is structurally very similar because a prospective lead compound's structure is 'fine-tuned' for better selectivity and potency toward the receptor of interest. Therefore, the mass spectrometric that analyses compounds in an analogous series quickly learns the most common metabolic alterations to the parent structure and any novel modifications. This experience and information allows for a guided analysis with targeted searches for expected metabolites. (To ensure that novel metabolites caused by less-common metabolic pathways are detected precursor ion scanning is available.)

9. Techniques and instrumentation:

An exhaustive description of all available MS instruments and their modes of operation is beyond the scope of this article and not necessary to appreciate the strategies being applied here. However, a basic knowledge of a few types of major systems and their important modes of operation is required and included.

9.1. Tandem MS: Tandem MS is the cornerstone of metabolite identification [12,14].Tandem MS actually covers a variety of scanning techniques including product ion, precursor ion, and neutral-loss scanning. Tandem mass spectrometers usually contain an isolation stage and a fragmentation stage within the same device. Although many different ways exist to complete a tandem MS experiment, all of them follow the same basic series of events. First, the ion of interest is isolated on the basis of its *m*/*z* ratio and then passed into the collision cell, a region of local high pressure. (In trapping instruments, the isolation and fragmentation normally take place within the same space between electrodes, and the stages are separated by time rather than space.) The collision cell is filled with an inert gas such as argon or helium, and a voltage is applied. Energized ions collide with the target gas, and each collision imparts a small amount of energy to the ion until sufficient energy is deposited to cleave an internal bond or bonds. The resulting ion fragments pass out of the cell and into the detector.

9.2. Targeted product ion analysis: In drug discovery, each new compound normally arises from a small alteration to an initial lead template that was discovered in a receptor-binding assay. The final compound may bear very little similarity to that initial lead, but it has a lineage that stretches back to the initial compound. Consequently, after the first one or two compounds have been fully assayed, any structural regions or 'soft spots' within the compounds in a drug series, which are highly susceptible to metabolism, are determined. Common metabolic alterations can be predicted, and a list of expected metabolites can be compiled, on the basis of a previously analysed series. By combining this list with a list of suspected metabolites identified by precursor and neutral-loss scan data, a series of ions can be targeted for production analysis.

Any type of mass spectrometer capable of product ion scanning can be used at this point, including a triple quadruple. Thus, all of the experiments discussed so far can be completed using a single instrument. However, to localize alterations within a molecule to a specific site, additional stages of tandem MS (MS) require a more specialized mass spectrometer.

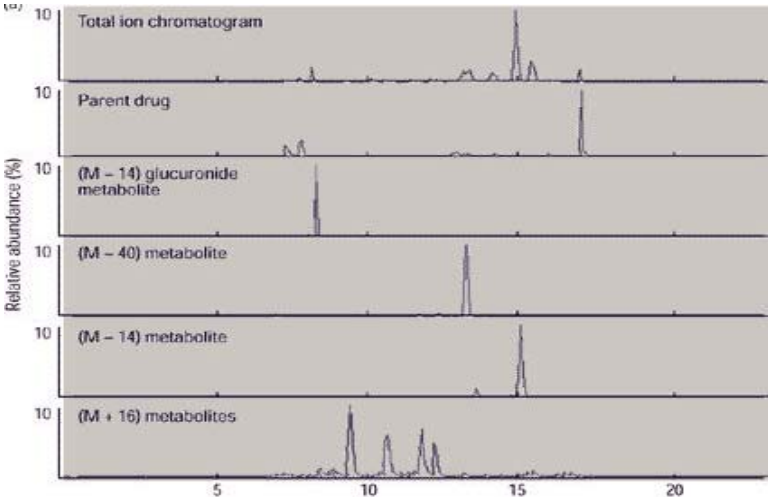
9.3. Determining sites of modification: Multiple stages of MS can provide large amounts of structural information regarding each analyse, thereby allowing for a more detailed characterization of the metabolites[15,16].

Completing MS experiments requires a mass spectrometer that can capture and store ions [13, 15, 16]. While the ions are stored, they can be subjected to excitation and collisional fragmentation. The trapping instrument can then capture the resultant fragment ions, which can then be forced to undergo further fragmentation. The second-generation mass spectrums will now give structural information regarding the isolated fragment, allowing easier characterization of that ion. Because this procedure can be applied to each of the initial parent ion fragments, detailed structural information can be acquired rapidly. This technique often allows the isolation of a small region of the parent ion molecule that has been modified and, in some cases, even the individual atom that are different. At present, the Fourier transform (FT) and the quadruple ion trap mass spectrometers are the only trapping mass spectrometers available FTMS instruments are not yet capable of high throughput on a regular basis because they are expensive and require skilled operators. Thus, cheaper and simpler quadruple ion trap mass spectrometers are typically used for these types of trapping experiments.

9.4. Identification of a dosed compound: With the basics of instrumentation and techniques described, a full metabolite identification study of a compound dosed in vivo can be examined. Sprague-Daley male rats were dosed intravenously with Schering-Plough discovery compound SCH X at 2 mg/kg body weight and orally at 10 mg/kg. Urine and bile were collected over 24 h at regular time intervals. Before analysis, the individual time point samples for each animal were pooled for each dosing region to generate one 0- to 24-h bile and one 0- to 24-h urine sample. No further sample preparation was performed. Minimal sample clean-up is used because the nature, number, and concentrations of metabolites present are unknown, and it is therefore impossible to determine if any will be lost during a sample preparation procedure.

9.4.1. Step 1: Collecting precursor ion scan and neutral-loss data: After sample preparation, a list of potential metabolites in the bile and urine sample needs to be compiled from precursor ion and constant neutral-loss analyses completed on a triple quadruple mass spectrometer. If the compound had been radio labeled, an online radioactivity detector would have also been used to mark time points where the compound elutes within the chromatographic run. Figure A demonstrates the type of data recorded in a precursor scan. At least eight apparent metabolites are evident in a single experiment, solely on the basis of the fragment ions' similarity to those produced by the parent compound standard. An additional five possible metabolites were found in the urine via precursor ion scanning data (data not shown). The total analysis took 44 min and required one bile and one urine injection to detect all 13 metabolites. Typically, more than a single series of fragment ions are scanned, corresponding to characteristic fragments from the top, middle, and lower portions of the parent compound. Common alterations to these fragments, such as hydroxylation, are monitored by looking for precursors of the analyse at the native fragment mass and fragments

Corresponding to the metabolic hydroxylations, which are 16 Da higher.



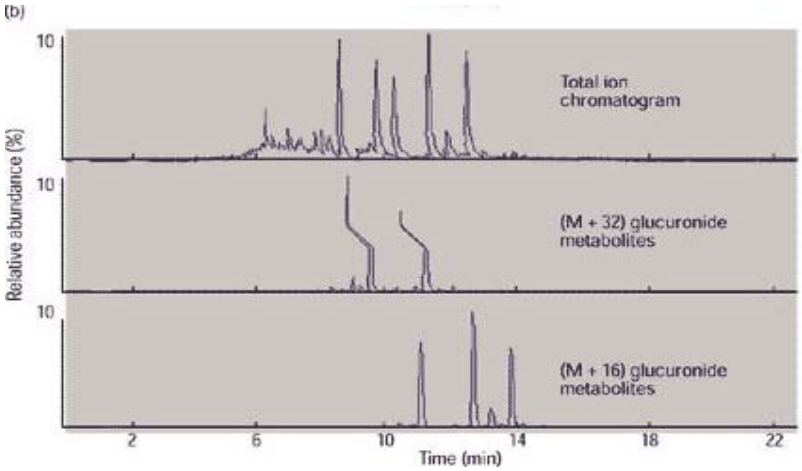
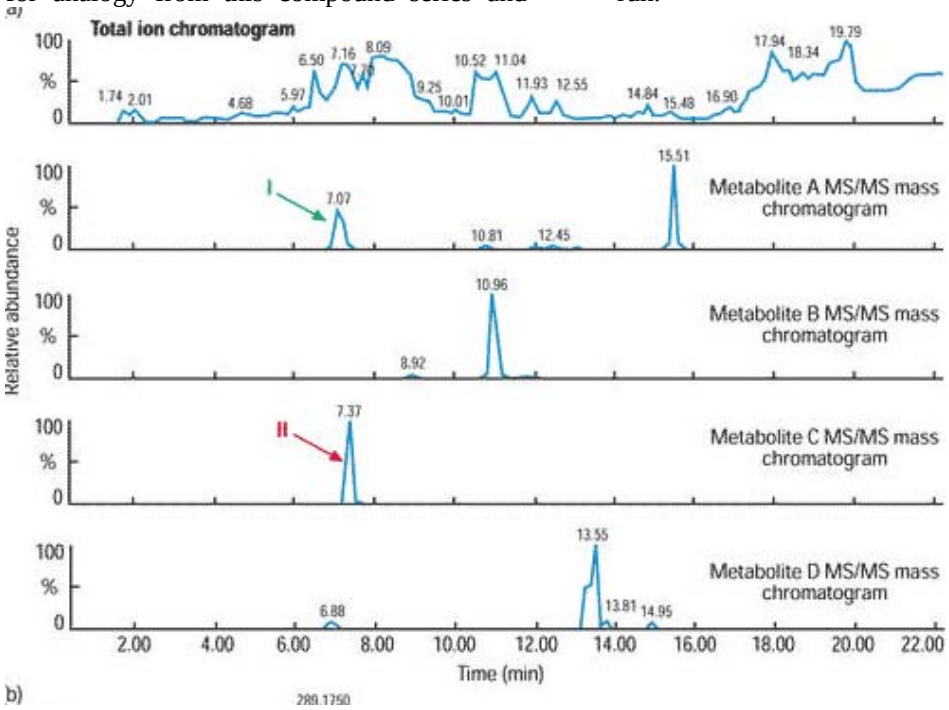


Figure.2: Precursor ion and constant neutral-loss scan experiments.
(a) Precursor ion scan and (b) constant neutral-loss (176 Da) scan experiments using rat bile. In both cases, the data are shown as an individual mass trace. Each trace is labeled with the putative metabolite's potential identity on the basis of their detected mass

9.4.2. Step 2: Product ion analysis of potential metabolites: The product ion data in the next step were acquired on the Q-TOF mass spectrometer, but a triple quadruple or quadruple ion trap mass spectrometer could also have been used. The Q-TOF instrument was used to examine a list of 'expected' metabolites that had been previously observed for analogy from this compound series and included+16 Da (hydroxylation), '14 Da (DE methylation), and +32 Da (hydroxylation). In addition, any putative metabolites identified by the precursor ion and neutral-loss scans also underwent product ion analysis. The rapid scanning abilities of the Q-TOF instrument allow several product ion experiments to be performed in rapid succession in a single LC run.



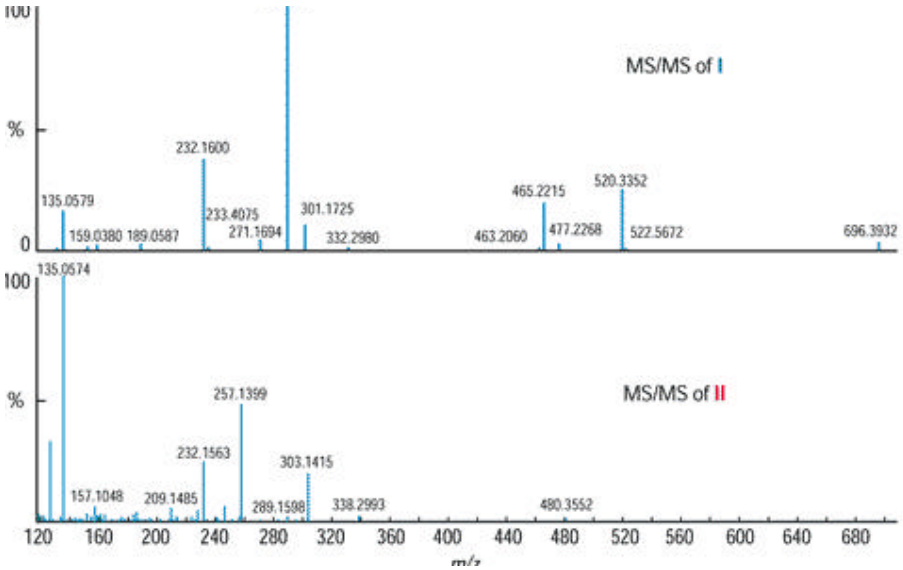


Figure.3. Product ion scan data used to identify several putative metabolites first detected in Figure 2. (a) Mass chromatograms for each of the examined metabolites. The individual traces are labeled with the potential identity of the metabolite on the basis of information from Figure 2 experiments. (b) Tandem MS spectra from peaks Ian II.

10. Structural elucidation of metabolites by MS.: Because of constraints due to space, expense, and complexity, the quadruple ion trap is the instrument of choice for MS experiments in a typical metabolite identification laboratory. For an MS experiment, the masses of the intact metabolite and a related fragment ion are required. Because this information comes from the MS/MS experiments described in Step 2, MS3 experiments can be set up directly on the instrument without any further experimentation.MS3 often gives data sufficient to determine the type of metabolite modification and indicate which parts of the molecule were changed. In most cases however, especially for molecules that only break into a few large fragments, MS4 or evenMS5 may be required to locate the site of modification, an O-glucuronide metabolite had to undergo MS5 before locating the site of oxidation. In each trace, the fragment ion that will undergo additional fragmentation is highlighted. Thus, this technique quickly pinpoints a very small area that has been altered.

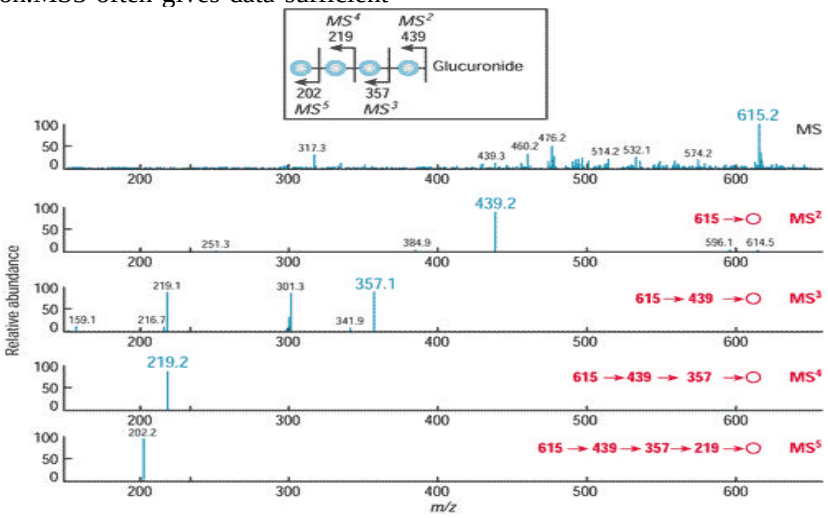


Figure.4.MS1 through MS5 data from the analysis of an O-glucuronide metabolite.
Each trace shows the next level of tandem MS in the sequential cleavage observed in the metabolite

10.1. Accurate mass measurement: The power of accurately determining the mass of a metabolite lies in being able to determine a list of possible empirical formulae. Obviously, the more accurate the mass measurements, the fewer degrees of freedom are available to the software calculating a formula, and the shorter the list of possibilities. Accurate mass product ion data further limits the number of possible formulae by providing data that are even more specific to the empirical formulae calculator. Product ion experiments narrow the site of modification to small portion of the molecule and an accurate mass determination of this fragment limits the software to fewer structural possibilities. Furthermore, the operator normally has prior knowledge of the parent compound's structure, which limits the number and types of atoms the software should take into consideration. Until recently, accurate mass measurement required double-sector instruments or Fourier transform ion cyclotron resonance instruments, which are very large, complex, and expensive. However, the advent of less complex and expensive bench top TOF instruments and hybrid instruments such as Q-TOF has allowed the application of accurate mass measurement in the metabolite identification laboratory.

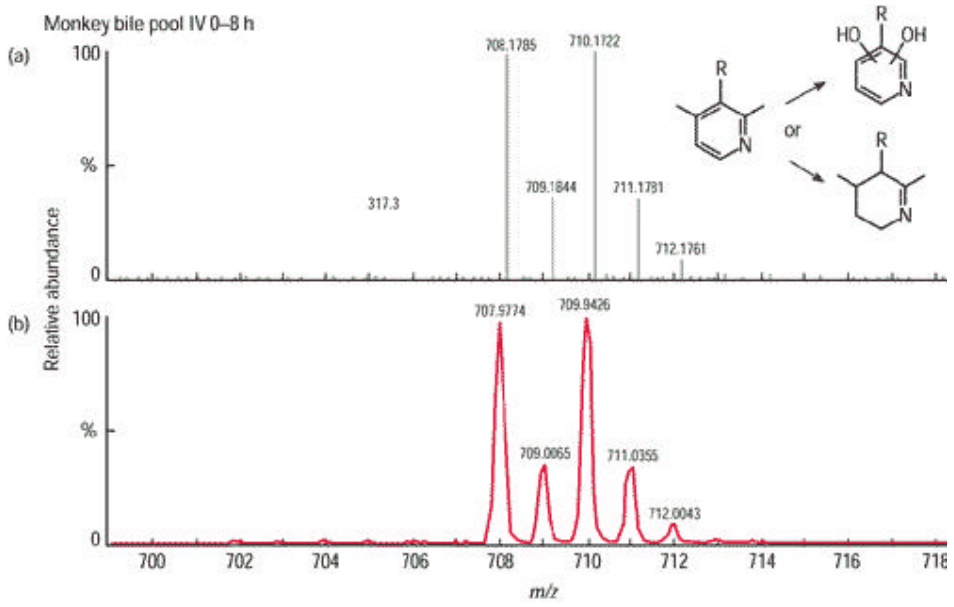


Figure.5.: Accurate mass data from monkey bile.
a) Raw data and (b) accurately Measured data after the external reference mass calibration was applied. The partial chemical structure shows the two most likely alterations that may have occurred

11. Still searching:
The strategies detailed in this report are the result of more than two years of work. They are not meant to exhaustively identify every metabolite present within *in vivo* samples, but rather to rapidly and accurately identify sites of metabolic liability that might affect pharmacokinetic measurement results and detect the formation of possibly toxic metabolites. Therefore, only major metabolites are identified, but the definition of a major metabolite differs widely among researchers. Because ~50%of our samples are radioactive, our laboratory definition of 'major' includes any resolved radioactive peak that constitutes 5% or greater of the overall radioactivity detected in that experiment. However, inevitably, other low-level metabolites are automatically detected using the prescribed strategies.

12. 10 years of MS instrumental developments – Impact on LC–MS/MS in clinical chemistry:
Within the past decade mass spectrometry (MS) has entered the clinical laboratory and is now being used for a wide range of applications. The technique can be considered essential for the determination of many clinically relevant analyses in combination with either gas chromatography (GC) or liquid chromatography (LC). The power of MS,

especially when coupled to LC, is recognized by clinical laboratories worldwide and the growing versatility of these systems puts clinical laboratories in a position where they can provide a rapid response to changing clinical needs. Even though it requires some effort much needed assays can be developed in the laboratory instead of waiting for a manufacturer to respond. Furthermore it is undoubted that these techniques pro-vide a higher level of sensitivity and specificity in many cases compared to other analytical techniques and that pateend care has benefited from their use. Besides specificity and sensitivity the ability of these techniques to measure multiple analyses simultaneously is a tremendous benefit of LC coupled to MS methods since many other techniques are limited to determine one analyse at a time. Especially these multi-component methods can make the purchase of a liquid chromatography tandem mass spectrometry (LC–MS/MS) instrument cost-effective. Improvements in automation and software help clinical laboratories to deal with staffing and service issues.

12.1. Ion source developments: Coupling MS to LC was a very important motivation in the development process of atmospheric pressure ion sources. Systems where the samples are introduced via a liquid stream achieved wide acceptance and commercial importance. Electro spray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the liquid introduction ion sources which had the most commercial success and enormous improvements were made in the first 15 years after their invention during the mid-1980s. Two excellent reviews were published dealing with the evolution of these ionization sources [16,17]. Based on these developments multimode ion sources were introduced on the market by various manufacturers. In addition the atmospheric pressure photo ionization (APPI) ion source was developed and improved within the last 10 years. Therefore more detailed information about multimode and APPI ion sources will be presented in the following chapters and their suitability regarding their application to clinical chemistry will be discussed. Apart these commercially successful LC–MS ion sources very creative approaches were investigated for the hyphenation of LC with MS using other available ion sources. Research scientists especially set their focus on various desorption techniques. The combination of LC with matrix-assisted laser desorption ionization (MALDI) started to emerge in the mid-1990s [18,19] and continuous effort was undertaken to further improve the technique [20–25]. Very recently LC–MS methods were described using desorption electro spray ionization (DESI) [26] and direct analysis in real time (DART) [27,28] interfaces.

12.2. Improvements in tandem mass spectrometry technology: Since LC–MS/MS technology is increasingly used for quantization in clinical science, as well as in other fields of science, there is a need for on-going improvements of the technology. A triple quadruple instrument in SRM mode is the instrument-of-choice in routine and high-throughput quantitative clinical analysis. Commercial triple quadruple MS with atmospheric pressure ionization (API) sources are widely used nowadays. In the case of triple quadruple instruments the most commonly requested improvements were defined by Bennett to be: greater sensitivity, dynamic linear range, mass resolution, wider mass range, faster acquisition cycle time and reduced cost of ownership. Advances in triple quadruple technology are challenging and focus remains in the source and interface regions to improve ruggedness and reduce matrix effects. Some minor improvements in quadruple manufacturing processes and RF power supply stabilities enabled the production of a commercial system with enhanced mass resolution without significant losses in ion transmission [51,52]. On the other hand significant instrumental developments were achieved in the last 10 years in the fields of other mass analysers like linear ion traps and HRMS like quadruple QTOF and FT-MS based instruments. Therefore the future of triple quadruples will be determined on the variable how extensively the clinical field adopts to high resolution, high mass accuracy instruments into their workflows and analytical requirements. Up to now, in case of triple quadruple instruments, mass resolution was typically ignored in favour of the outstanding linearity and increased sensitivity due to the selectivity offered by tandem MS. Now new tasks are gaining more and more interest where improved selectivity and full-scan data at low duty cycle times are crucial.

13. Schematic of a quadruple linear ion trap and description:

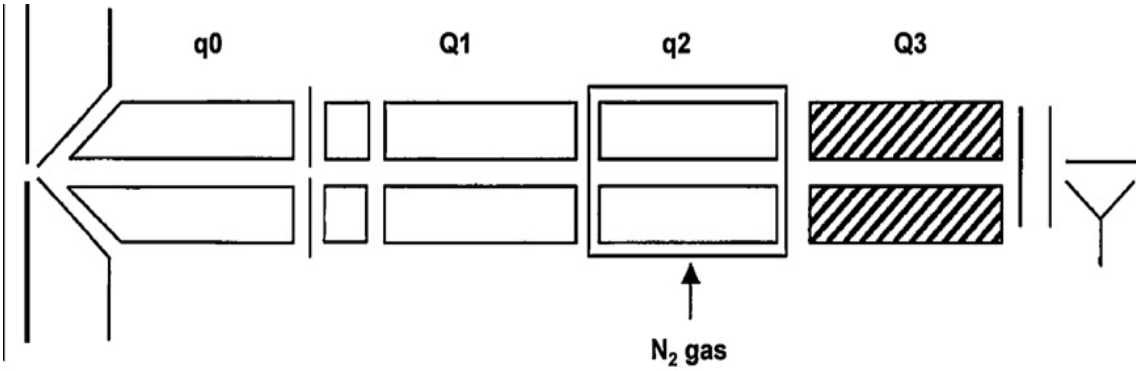
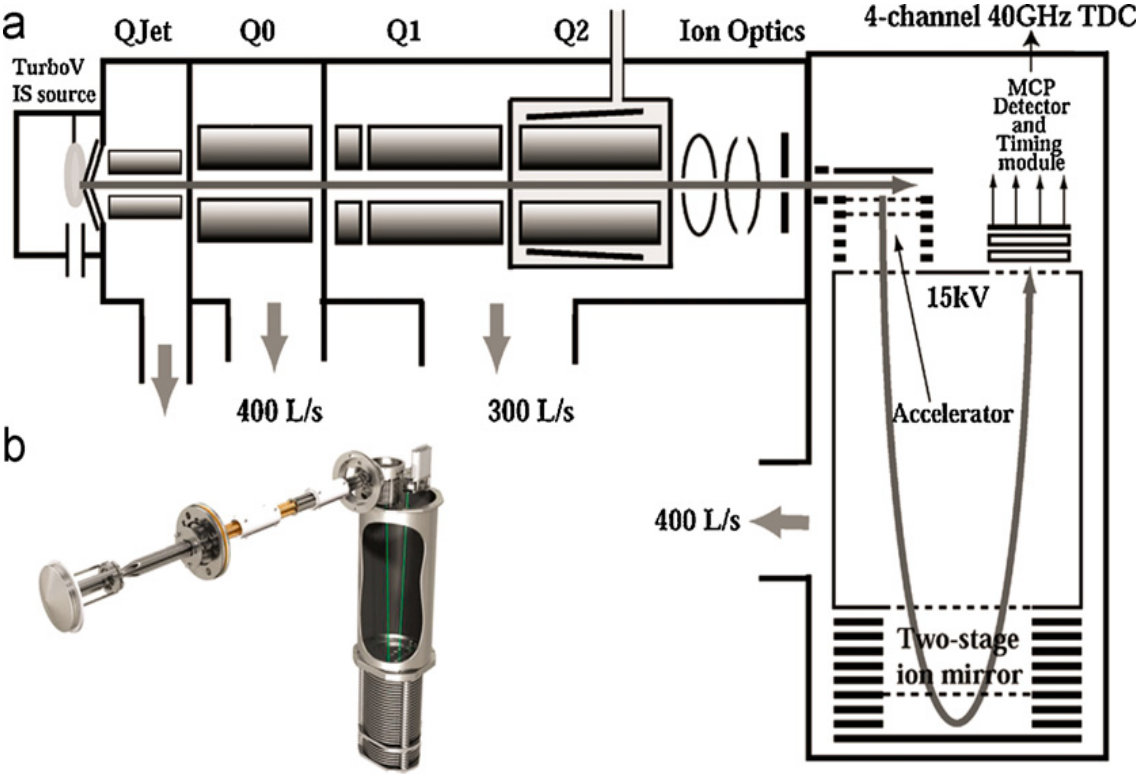
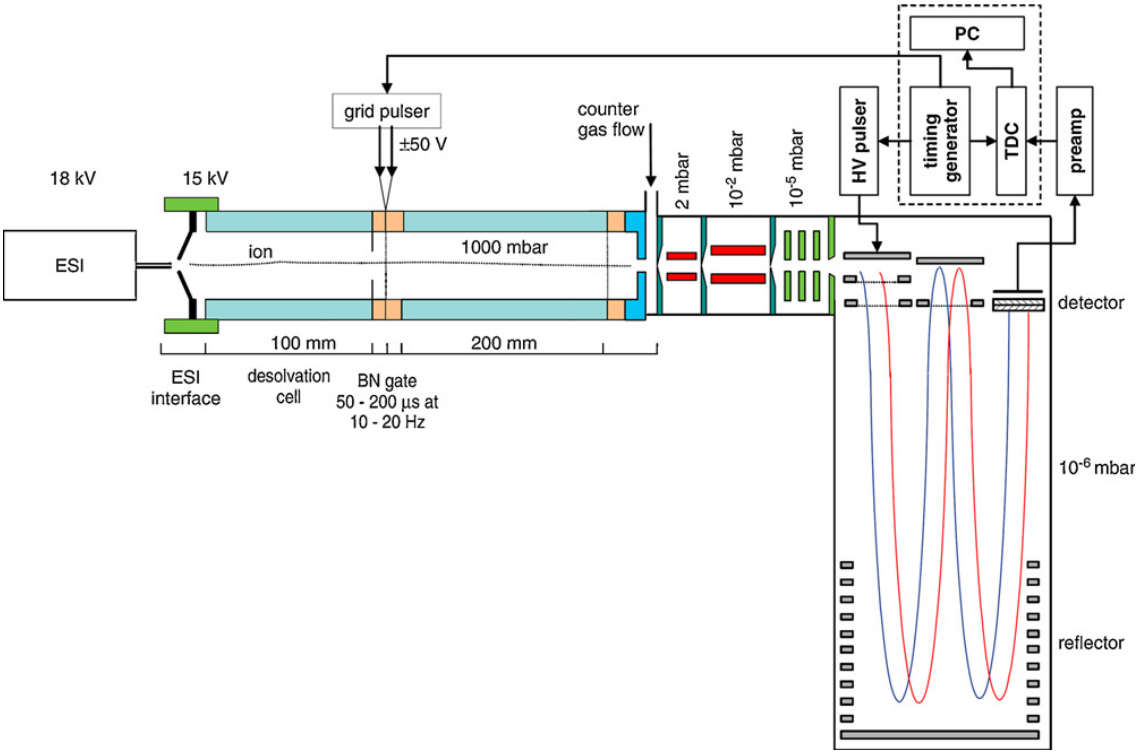


Figure.6:TheTriplet OF MS technology features diagrammed.
(a) A detailed illustration of the major platform features. (b) An image of the machined Triplet OF MS instrument platform**.**



Ion mobility-mass spectrometry: Interfacing ion mobility spectrometry (IMS) with MS can provide significant advantages. The potential was understood early in the development of IMS, and the coupling of the two techniques is virtually as old as IMS itself. So ion mobility-mass spectrometry (IMMS) cannot be regarded as new, but after the demonstration of protein conformer separation by Calmer *et al*. [1] there was a considerably increase in interest within this research area. During the last 10 years instruments became commercially available and both applications and instrumental designs of IMMS are now one of the most rapidly growing areas of MS. As a matter of fact numerous articles, reviews and even books} are dealing with the topic of IMMS.

Schematic of an ambient-pressure IMS(toff)MS.



14. Progress in automation of LC-MS in laboratory medicine:

Standard techniques of analyse detection in clinical chemistry rely on indirect characteristics of an analytic, e.g. its absorption of light, chemical reactivity or physical interaction with macro-molecules. In mass spectrometric methods, in contrast, analyses are detected directly from molecular characteristics as molecular mass and molecular disintegration patterns. Thus, mass spectrometric techniques are very attractive for the quantification of biomarkers or xenobiotic in the context of diagnostic procedures, since those techniques can enable analyses of much higher specificity compared to standard technologies such as photometry or ligand binding tests. With gas chromatography–mass spectrometry (GC–MS), first mass spectrometric methods were introduced to laboratory medicine about 40 years ago. GC–MS allowed the highly specific and sensitive quantification of thermo-stable molecules below a molecular weight of about 500. Thus, GC–MS became a key technology, e.g. in toxicology. With respect to standardization and quality assurance of small molecule analytical routine methods the introduction of GC–MS as a reference method was an essential progress, in particular for endocrinology. However, for several reasons the application of GC–MS remained restricted to few specialized institutions in laboratory medicine (mainly toxicological laboratories, metabolism centres, and reference laboratories). The handling and maintenance of GC–MS instruments is very demanding and time-consuming; sample preparation is very laborious and includes sample extraction and analytic reprivatisation; the analytical run times are long with a typical sample throughput of less than 50 samples per day

15. Future of LC-MS/MS application in laboratory medicine:

At present only rather few clinical laboratories worldwide are equipped with LC-MS/MS systems, and in these laboratories this technology typically makes up for less than 1% of all analyses. The success of efforts to substantially improve the practicability and

robustness of LC-MS/MS application by automation will be crucial for a more widespread application of this technology in the future. But is application of LC-MS/MS in laboratory medicine beyond the status quoi really reasonable? Indeed LC-MS/MS can close substantial gaps in the parameter portfolio of laboratory medicine

LC-MS/MS can allow routine analyses on a reference method level of accuracy (incorporating isotope dilution technology) for important analysts for which immunoassays offer critically limited accuracy or cannot be applied at all (e.g., steroid hormones , plasma metanephrins, 25-hydroxyvitamin D, drug of abuse testing; methyl malonic acid, asymmetric dimethyl arginine, microbial antigens).

LC-MS/MS can allow really comprehensive therapeutic drug monitoring (including assessment of metabolisation) for a personalized drug therapy; this is of utmost importance in the context of recent findings of pharmacokinetics for a large variety of drugs. The availability of companion testing will probably also becomes a key issue in the licensing of new drugs.

LC-MS/MS enables highly multiplexed metabolic profiling which probably holds substantial potentials for disease monitoring. LCMS/ MS will probably be the analytical platform for all analyses which will be discovered to be useful in the context of metabolomics research.

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