The use of high-performance liquid chromatography combined with mass spectrometry or tandem mass spectrometry has proven to be the analytical technique of choice for most assays used in various stages of new drug discovery

Foundation review:

Principles and applications of LC–MS in new drug discovery

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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.

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 [1–10]. 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. Furthermore, there are also a series of drug metabolism and pharmacokinetics (DMPK) tests that are performed as part of new drug discovery; these tests measure the absorption,

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Walter A. Korfmacher is a Director of Exploratory Drug Metabolism at Schering-Plough Research Institute in Kenilworth, New Jersey. He received his BS in Chemistry degree from St. Louis University in 1973. He then went on to obtain his MS in Chemistry in 1975, and PhD in Chemistry in 1978, both from the University of Illinois in Urbana. In 1978, he joined the FDA and was employed at the National Center for Toxicological Research (NCTR) in Jefferson, Arkansas. While at the NCTR, he also held Adjunct Associate Professor positions at the College of Pharmacy in the University of Tennessee (Memphis) and the Department of Toxicology in the University of Arkansas for Medical Sciences (Little Rock). After 13 years at the NCTR, Korfmacher joined Schering-Plough Research Institute as a Principal Scientist in October 1991. He is currently a Director and the leader of a group of 14 scientists. His research interests include the application of mass spectrometry to the analysis of various sample types, particularly metabolite identification and trace organic quantitative methodology. His most recent applications are in the use of HPLC combined with atmospheric pressure ionization mass spectrometry and tandem mass spectrometry for both metabolite identification and nanogram per ml quantitative assay development for various pharmaceutical molecules in plasma. He is also a leader in the field of developing strategies for the application of new MS techniques for drug metabolism participation in new drug discovery and is frequently invited to speak at scientific conferences. In 1999–2000, Korfmacher was the chairperson of the North Jersey Mass Spectrometry Discussion Group and, in 2002, received the New Jersey Regional Award for Achievements in Mass Spectrometry. Korfmacher is a member of the editorial board for *Rapid Communications in Mass Spectrometry*; he has also edited a book entitled *Using Mass Spectrometry for Drug Metabolism Studies* (CRC Press, 2005), has over 100 publications in the scientific literature and has made over 75 presentations at various scientific forums.

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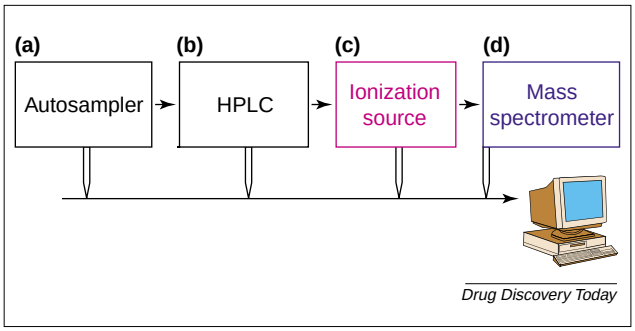

FIGURE 1

The elements of an LC–MS system. (a) Autosampler (loads the samples onto the HPLC); **(b)** HPLC; **(c)** ionization source (interface for LC to MS); **(d)** Mass spectrometer (various types, see Figure 2).

distribution, metabolism and excretion (ADME) properties of the NCE, as well as the pharmacokinetic (PK) parameters of the molecule. Most of these assays rely on HPLC–MS or HPLC–MS–MS for the measurement step.

This review will provide an overview of the various ways in which LC–MS (which will be used as a term that includes both HPLC–MS and HPLC–MS–MS) can be used in the new drug discovery process. The 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.

Principles of LC–MS

As shown in Figure 1, the elements of an LC–MS system include the autosampler, 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 analytes 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 [1,11–13].

There are various types of ionization sources that can be used as the interface between the HPLC eluant and the mass spectrometer. The two most common sources are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI); both of these source types are now standard equipment on mass spectrometers that are used for LC–MS applications. For both ESI and APCI, the

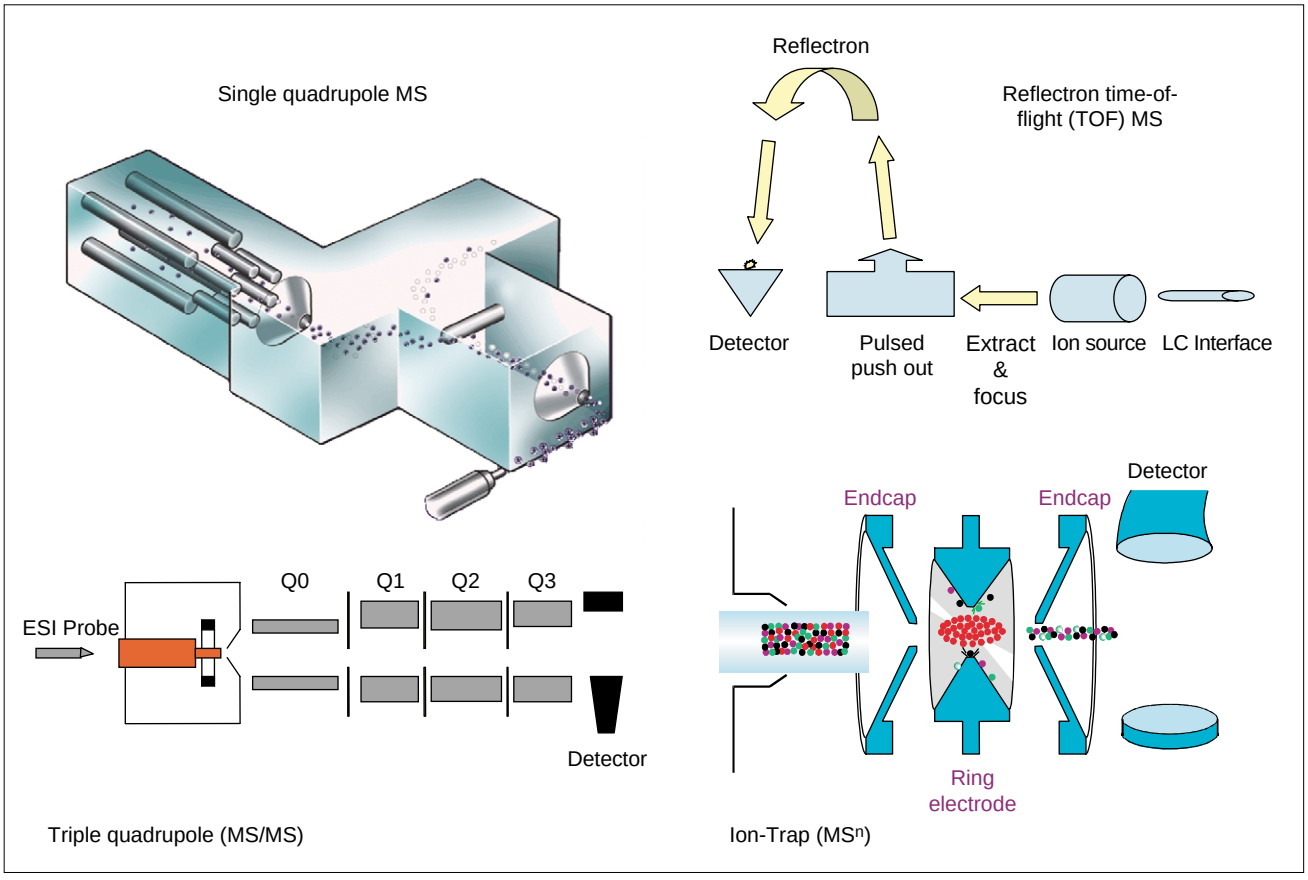

FIGURE 2

Types of mass spectrometers that can be used in LC–MS systems. Figure adapted, with permission, from Ref. [3].

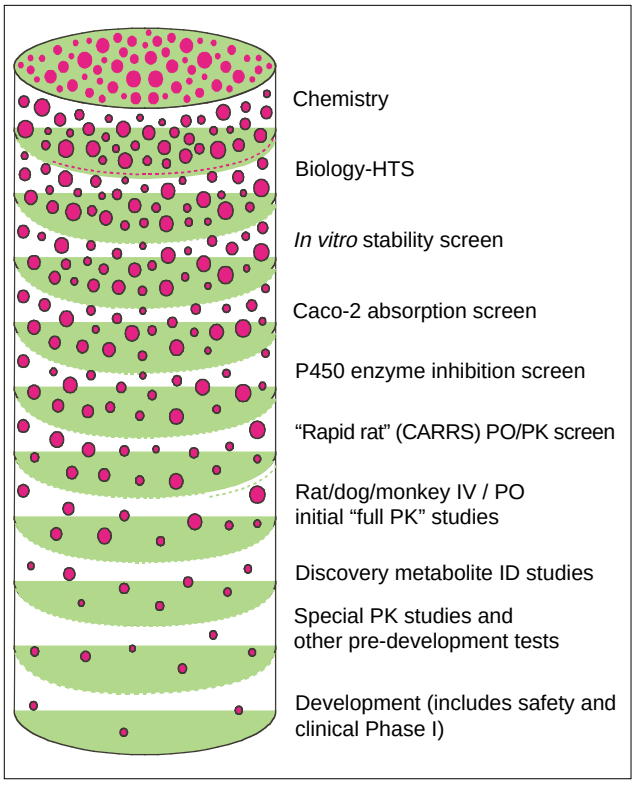

FIGURE 3

Stages of new drug discovery from the initial synthesis to Phase I in humans. Figure adapted, with permission, from Ref. [3].

ionization occurs at atmospheric pressure, so these sources are often referred to as atmospheric pressure ionization (API) sources. For both ESI and APCI, some combination of high voltage and heat is used to provide the ionization that is needed to produce the ions that are assayed by the MS system. In ESI, the high voltage field (3–5 kV) produces nebulization of the column effluent resulting in charged droplets that are focussed toward the mass analyzer. These droplets get smaller as they approach the entrance to the mass analyzer: as the droplets get smaller, individual ions emerge in a process referred to as 'ion-evaporation' – these ions are then separated by the MS system [4,14]. In APCI, heat is used to vaporize the column eluant and then a corona discharge is used to ionize solvent molecules, which then produce the analyte ions via chemical ionization mechanisms [4,14]. More recently, a third type of ionization source, termed atmospheric pressure photoionization (APPI), has become available [14–18]. In APPI, heat is used to vaporize the column eluant (similar to APCI) but the ionization is produced by way of an ultraviolet (UV) lamp that produces 10 eV photons. Depending upon the solvent system used, the 10 eV photons will either ionize the mobile phase solvent or a dopant (a compound such as toluene that can be ionized by the 10 eV photons) added to the column effluent; these ions then produce the analyte ions through various ionization mechanisms including charge or proton transfer [14,19–21].

Types of mass spectrometers

There are many types of mass spectrometers available for interfacing with HPLC. As shown in Figure 2, one of the more common systems used for HPLC–MS is the single quadrupole mass spectrometer; this system will provide a mass spectrum for each chromatographic peak that elutes from the LC column and is analyzed by the MS system. The second type of system shown is the time-of-flight (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 quadrupole MS–MS system, which is most often used for bioanalytical assays but can also be used for metabolite identification assays [1,3,22,23]. The fourth MS system is called an ion-trap mass spectrometer and has the unique capability of producing MS^n data that are important when performing structural elucidation assays [22,23]. 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 quadrupole mass spectrometer with a TOF mass spectrometer [22,23]. In the following discussion on uses of LC–MS in new drug discovery, references will be made to these various types of mass spectrometers and their ionization source when necessary.

Stages of new drug discovery

There are various stages in new drug discovery, from chemical synthesis to compound selection for development, as shown in Figure 3. LC–MS has been used to support all of these stages and has become the preferred analytical tool in many cases. In the following sections, one or more examples of how LC–MS has been used for the specific type of assay needed for that stage will be shown.

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 quadrupole 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. Simpler HPLC–MS systems have been described to

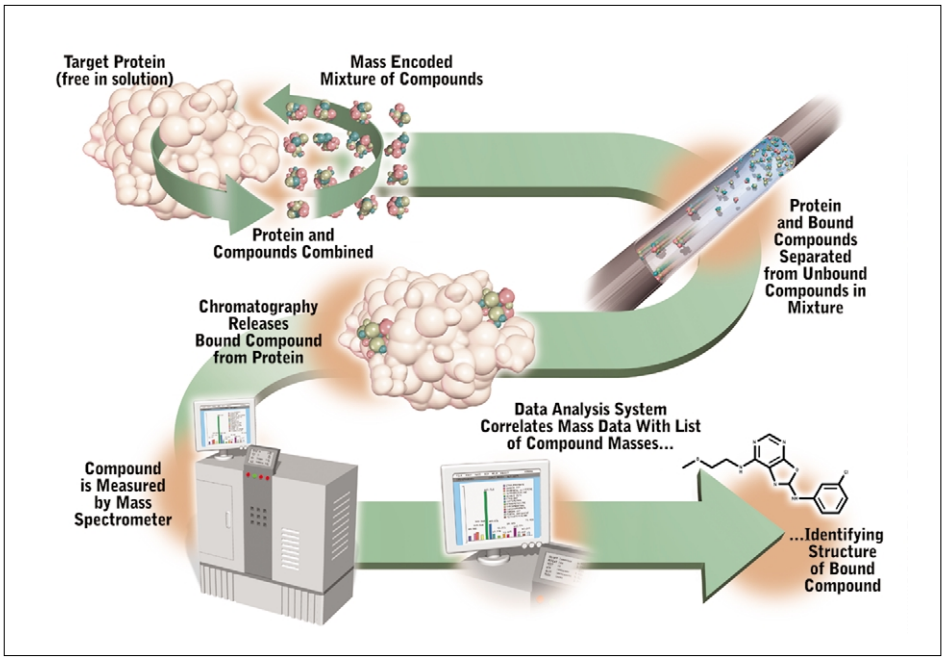

FIGURE 4

Diagram showing how the Automated Ligand Identification System (ALIS) system uses MS for high throughput activity screening. The figure was provided by Satish Jindal and Allen Annis from Schering-Plough-Cambridge (Cambridge, MA, USA) and used with their permission.

provide an 'open-access' environment so that medicinal chemists can easily use HPLC–MS to monitor their chemical reactions. For example, Mallis *et al.* [25] have described how an open-access HPLC–MS system based on a single quadrupole mass spectrometer has been used for aiding medicinal chemists as part of new drug discovery.

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 (for an example, see Figure 4). As discussed by Falb and Jindal [26], the strategy uses mixtures of compounds plus a target protein to identify potential lead compounds for a therapy based on compounds 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 MS.

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]. As shown in Figure 3, 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. The following sections will show examples and references for these assays.

In vitro *screening*

One of the more commonly used *in vitro* screens is the human colon adrenocarcinoma 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 [31–33]. There are several reports on how LC–MS can be used for the analysis of Caco-2 samples [34–38]. 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 quadrupole 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 Feng *et al.* [35] 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 the 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 [33,39,40]. 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 [2,41–44]. In a recent example, Peng *et al.* [43] described a high throughput assay based on HPLC–MS–MS to screen for five important CYP isozymes – CYP 3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2. Their method was based on a human liver microsomal incubation of six CYP-specific probe substrates. The samples were analyzed via HPLC–MS–MS (based on a triple quadrupole MS system) using a monolithic silica rod column that allowed for a ballistic gradient and a mobile phase flow rate of 5 ml per min, and a total sample run time of 24 s. To keep the ionization source cleaner, the authors reported that for the first 12 s of the sample elution time, the column effluent was diverted to waste. This is a good practice for any high throughput

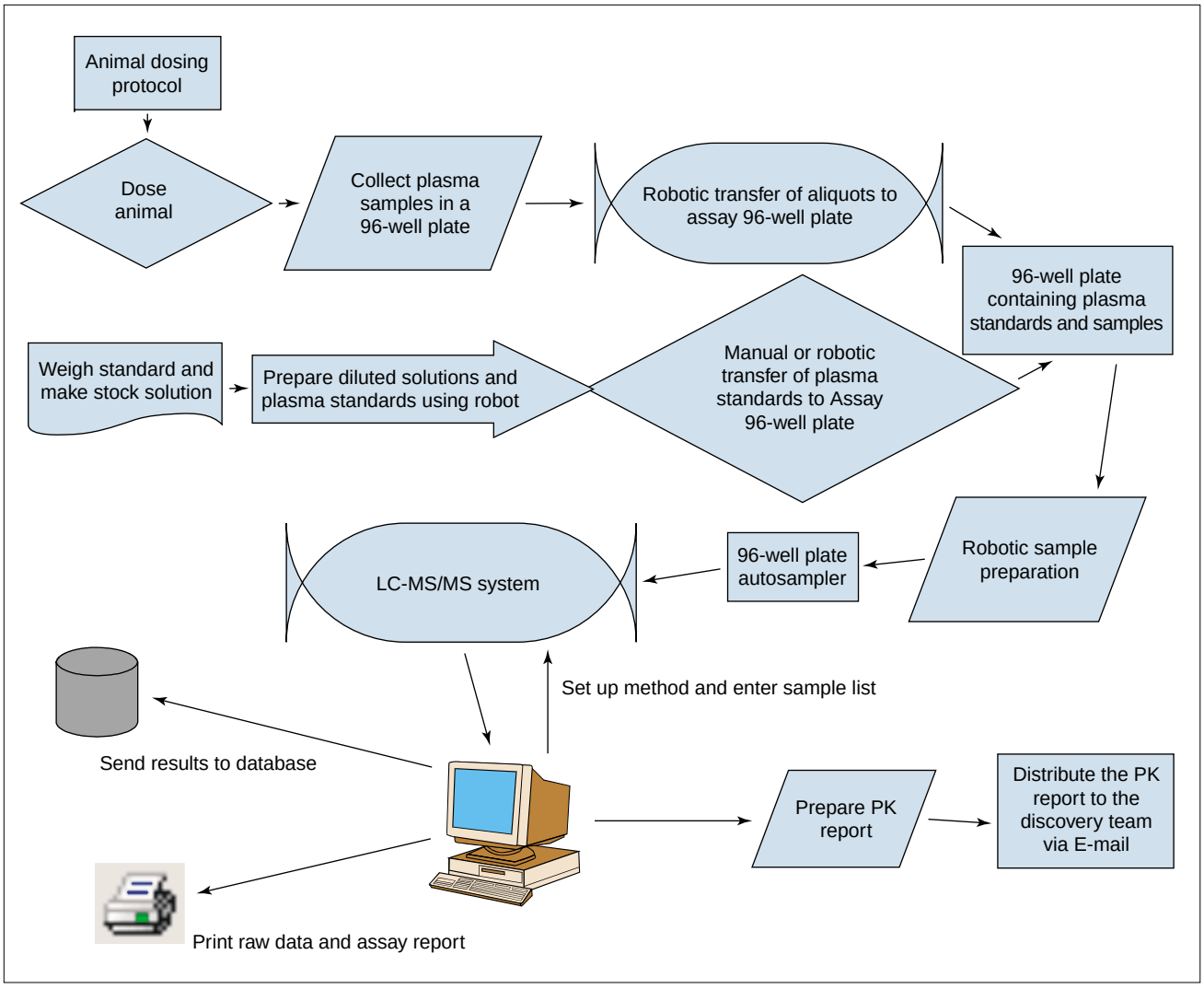

FIGURE 5

Flow chart for discovery PK assays using HPLC–MS–MS. Figure adapted, with permission, from Ref. [3].

assay based on LC–MS – divert the first 20% (or more) of the column effluent to waste so that only the portion of the chromatogram that includes the analytes is sent to the MS system.

A third important *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 [33,45]. Ansede and Thakker 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 [46]. Generally, the metabolic stability assays are based on the incubation of the compound in the presence of either human liver microsomes 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 quadrupole system). Several examples of high throughput metabolic stability assays based on LC–MS have been reported [2,45,47–56]. In an early example, Korfmacher *et al.* [51] described an automated assay based on a single quadrupole LC–MS system that used an automated data analysis system and could test 75 compounds per week for metabolic stability. Wring *et al.* [56] described a system for metabolic stability that included automated liquid handling and an LC–MS assay based on a triple quadrupole mass spectrometer that was capable of handling 50 compounds per week. More recently, Xu *et al.* [50] 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, 15 and 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 quadrupole LC–MS system that included an eight-probe autosampler and eight HPLC columns in a parallel mode. This system was able to assay 240 samples per hour, which enabled up to 176 test compoundsto 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.

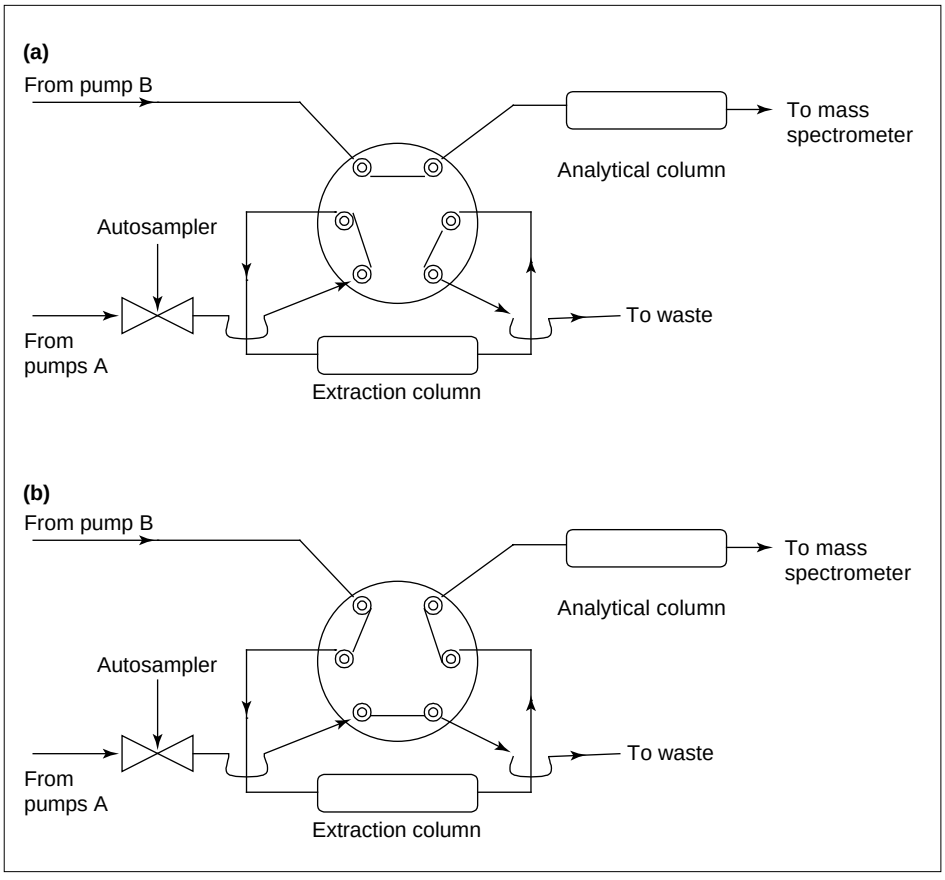

FIGURE 6

The flow paths used for an online extraction system. (a) Configuration for loading sample, extraction and equilibriation. **(b)** Configuration for elution. Figure adapted, with permission, from Ref. [64].

In vivo *PK screening*

Although the various *in vitro* assays are generally higher throughput, the *in vivo* screening assays are typically much lower throughput. The multiple steps involved in a typical discovery PK assay are shown in Figure 5. The most common type of mass spectrometer for these assays is the triple quadrupole mass spectrometer system. Recent review articles by Hopfgartner and Bourgogne [57] and Cox *et al.* [58] provide some examples of the various creative ways some groups have devised for improving the throughput of these *in vivo* screens. Most of the methods for increasing throughput have focussed on one of the following: sample preparation strategies, sample reduction schemes or faster HPLC systems.

Various sample preparation strategies have been implemented to improve the throughput of the *in vivo* PK screens. As shown in Figure 5, one of the most common strategies is to use 96-well plates for the sample preparation steps. For discovery PK assays, protein precipitation is often used as the sample preparation step [3,5,27,57,59,60]; this technique has the advantage of speed and can be performed in a semi-automated manner using various robotic devices, such as the Tomtec Quadra 96® liquid handler system [3,59]. For development compounds, it would be more common to use either liquid-liquid extraction (LLE) or solid-phase extraction (SPE) for the sample preparation step; these procedures have the advantage of typically providing a cleaner extract but a disadvantage is that some method development and optimization is usually required for each compound to be assayed [10,61]. In addition to off-line sample preparation techniques, there have been multiple reports describing various on-line techniques, which are often referred to as direct plasma analysis methods [62–72]. For example, Jemal *et al.* [65] described the utility of a typical direct plasma injection system that uses an extraction column to separate the analyte from the plasma matrix components followed by an analytical HPLC column that separated the analyte from some of the other retained sample components and eluted into the MS system (see Figure 6). In this report, Jemal *et al.* [65] compared the results from direct plasma injection system to the same assay based on LLE; the results of the two procedures were comparable but the direct injection procedure required less time for the sample preparation step. One drawback to this type of on-line system is that it is serial in nature – the extraction step needs to be performed before the analytical step. Xia *et al.* [66] improved upon this concept by designing a parallel system so that while one sample is being extracted, another sample is going through the analytical column. In a similar manner, Hsieh *et al.* [72] described a dual extraction system based on turbulent flow columns connected to dual analytical columns as part of a validated method designed to meet good laboratory practices (GLP) criteria. Hsieh *et al.* [63] showed a different approach in which a single mixed function column could be used for both the extraction step and the analytical step in a direct plasma assay procedure.

Sample reduction schemes have been based on either a sample pooling process or on cassette dosing procedures [3,57,58]. Hop *et al.* [73] described one version of a sample pooling procedure in which multiple timepoint samples were pooled into one sample to reduce the number of samples to be assayed; using this procedure, an area under the curve (AUC) estimate was obtained for each animal that was dosed. Hsieh *et al.* [74] described a cassette analysis procedure in which samples from individually dosed animals were pooled to reduce the number of samples to be assayed. As LC–MS assays have become faster (*vide infra*) and more highly automated, there has been a reduced need for these sample-pooling strategies.

Another popular procedure for sample reduction has been cassette dosing. In cassette dosing (also called

N-in-one dosing), multiple (typically 5–10) compounds are dosed in the same laboratory animal [6,58,75–78]. Because HPLC–MS–MS systems can be used to assay multiple compounds in one analysis, cassette dosing offered the possibility of obtaining more information from fewer samples. Unfortunately, as discussed by White and Manitpisitkul, cassette dosing can also lead to both false positives and false negatives when it is used to determine the PK parameters of new discovery compounds [79]. Despite these problems, the authors recently reported that ~50% of the major pharmaceutical companies that they surveyed still use cassette dosing for at least some of their discovery programs [78]. As an alternative to cassette dosing, Korfmacher *et al.* [59] described a procedure called the cassette-accelerated rapid rat screen (CARRS), in which cassettes of six discovery compounds are dosed into 12 rats (two rats per compound); although CARRS is not a cassette dosing procedure, it is a cassette assay procedure in that each set of compounds is assayed in one analytical procedure using an HPLC–MS–MS (triple quadrupole) system to speed up the compound throughput. In the CARRS report, the AUC for the first six hours post-dose is reported, as well as six individual timepoints that provide an indication of the oral PK parameters for the test compounds. As reported recently, the CARRS system has been used as a rat oral PK screen for over 7000 discovery compounds in the past several years [78].

Faster LC–MS systems have also become a common method for speeding up the throughput of discovery PK assays [11]. Various strategies have been used to speed up the HPLC part of the HPLC–MS system; one common strategy being to use smaller HPLC columns [80–82]. For example, Romanyshyn *et al.* discuss the importance of using fast gradients (versus isocratic chromatography) and demonstrate that a method for a discovery PK assay can be developed easily with a 2.0 min gradient on a 2 x 20 mm HPLC column [80]. Another common approach is to increase the mobile phase flowrate as a way to speed up the LC–MS assay [83,84]. For example, Hsieh *et al.* [83] demonstrated that, by using a monolithic HPLC column and an increased flow rate, discovery compounds for PK studies could be assayed in less than one minute per sample. Deng *et al.* [84] described a system in which four monolithic HPLC columns were operated in parallel to achieve a throughput of 30 s per sample in an HPLC–MS–MS assay.

As test drugs become more potent, it is important to be able to get PK results from studies where lower amounts of the test compound are given to the laboratory animal. This means that lower limits of quantitation (LOQs) are needed to assay these samples. To meet these needs, instrument vendors have provided more sensitive triple quadrupole mass spectrometers in recent years. A new problem that has arisen as lower LOQs are achieved is that it is becoming more common to see interferences despite the high specificity that is provided by LC–MS–MS systems when used in the selected reaction monitoring (SRM) mode that is used when triple quadrupole MS systems are used for quantitative assays [85–87]. Recently, one vendor has developed a triple quadrupole mass spectrometer with an enhanced mass resolution capability [85–87]. The enhanced mass resolution has been found to be useful in helping to reach lower LOQs, particularly in a drug discovery setting [85–87]. In a typical drug discovery PK assay, the minimal plasma sample cleanup provided by the protein precipitation procedure can lead to signals from endogenous components at the 0.1–0.5 ng per ml levels that are sometimes needed as the LOQs for these assays; the enhanced mass resolution capability can often eliminate these interfering signals and enable the assay to be used at these LOQs [85–87].

Matrix effects

As LC–MS assays became faster, a problem emerged that had to be monitored more carefully – matrix ion suppression (also known as 'matrix effects'). 'Matrix effects' is a term that has been used to describe any change in the MS response of an analyte that is a result of the specific matrix of the sample being assayed. It is important to realize that matrix effects can lead to either a reduced response (ion suppression) or an increased response (ion enhancement) of the MS system [88,89]. As users of LC–MS systems become more familiar with matrix effects and how to avoid them, this potential problem will be avoided in most cases.

One of the best tools for observing matrix effects is the post column infusion technique [88,89] (Figure 7). Typically, the analyte of interest is infused at a steady rate into the HPLC eluant stream that is directed into the MS system. First, a sample of mobile phase or a solvent blank is injected and the LC–MS assay is conducted as normal to provide the baseline for comparison. Then a control sample extract is injected and the LC–MS assay is conducted.

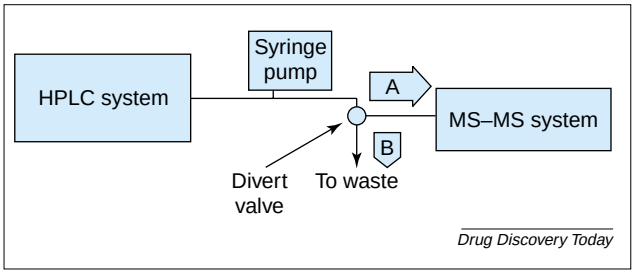

FIGURE 7

The procedure for conducting the post-column infusion experiment that can be used to detect matrix effects in an HPLC–MS–MS assay. Procedure: (1) inject mobile phase or solvent blank while infusing analyte into the column eluant at a flow rate of 5–10 μl/min and follow path A. (2) Inject control sample extract while infusing analyte into the column eluant at a flow rate of 5–10 μl/min and follow path A. (3) Compare the mass chromatograms – differences are due to matrix effects. To analyze the samples, inject sample (syringe pump off) and divert the flow to waste (path B) for the first 10–25% of the assay run time, then switch the divert valve to send the eluant into the MS–MS system (path A) for the analysis of the sample.

By comparing the two results, one can see the times in the mass chromatogram that have matrix effects – typically, this will only be during the first 20–25% of the HPLC gradient time for a well designed method. Indeed, a common procedure is to divert the first 10–20% of the sample runtime to waste so that only the 'cleaner' part of the HPLC eluant will enter the MS system (see Figure 7).

One of the most important tasks for the HPLC system is to separate the analyte from those components of the sample that can cause matrix effects. Once the region of matrix effect is known for an assay, then it is just a matter of selecting the 'matrix-effect free' portion of the chromatogram for the analysis. Tiller and Romanyshyn [90] described how an HPLC gradient procedure can be used to avoid the matrix effect problem and also to provide faster assays. For example, Hsieh *et al*. [91] described how the post column infusion technique could be used to ensure that even with a fast HPLC gradient, the analyte can elute during a matrix-effect free region of the chromatogram and a valid HPLC–MS–MS assay can be performed. Another consideration when dealing with matrix effects is the choice of APCI versus ESI. APCI is generally considered to be less susceptible to matrix effects compared with ESI [89], although recent reports have shown that APCI can also display matrix effects in some cases [88,92,93].

Metabolite identification

Metabolite identification has become an integral part of the drug discovery process [94] and one for which LC–MS continues to be an important tool [5,22,23,94–97]. Although metabolite identification can be performed solely with a triple quadrupole mass spectrometer as described by Korfmacher *et al*. [5], it is preferable to use additional types of mass spectrometers [23,95]. A triple quadrupole excels at finding the metabolites through a combination of various scan functions, including MS and precursor ion scanning, as well as constant neutral loss scanning [5,23,95]. Once metabolite peaks have been identified, the triple quadrupole can also provide product ion scanning to produce a product ion mass spectrum that can be compared to that of the dosed compound (in an *in vivo* study) and this can often lead to one or more possible structural assignments for the metabolite. An ion trap LC–MS system can be used to provide MS^n analysis of a metabolite and this can be used in some cases to refine the structural assignments so that a smaller set of possibilities emerges [23,95]. A hybrid Q-TOF MS–MS system is a high sensitivity tool for low level metabolites and can provide an important additional set of data – exact mass of the metabolite or of its product ions [23,95]. This information can often be used to select the one metabolite structure with the empirical formula that corresponds to the measured exact mass.

Various manufacturers of MS–MS systems now provide metabolite identification software tools that aid in finding and identifying metabolites [23]. In addition, software tools that can be used to 'sift' through complex LC–MS data have become available recently. One such tool is the isotope cluster analysis tool that can be used when the dosed compound (and therefore most of its metabolites) has a distinctive isotope ratio in its mass spectrum (e.g. compounds containing one or more Br atoms) [23]. In future, it is probable that additional software tools will become available to aid in the metabolite identification process.

There are also new types of mass spectrometers that have become available in the past few years that have great utility for metabolite identification. One such tool is the Q-Trap MS–MS system. The Q-Trap MS–MS system combines the utility of a triple quadrupole mass spectrometer with the special capabilities of a linear ion trap system. Recently, Hopfgartner *et al*. [98,99] described the special capabilities of the Q-Trap MS–MS system in detail and have shown examples of its use in metabolite identification. Another new instrument is the linear ion trap–fourier transform mass spectrometer (LTQ–FTMS) system; this system combines the capabilities of a linear ion trap MS system with the high mass resolution capabilities of an FTMS system. A new chromatographic tool, ultra-high performance liquid chromatography (UPLC) has also become available recently; UPLC uses small particle packing column material and high pressure pumps to provide higher chromatographic resolution capabilities. UPLC clearly has great potential for metabolite identification applications and references to UPLC–MS can now be found in the literature [100,101]. As these new systems become more common, they will become important tools for scientists involved in metabolite identification efforts.

Conclusions

HPLC–MS and HPLC–MS–MS continue to be the premier analytical tools for most *in vitro* and *in vivo* DMPK assays that are part of new drug discovery. HPLC–MS and HPLC–MS–MS have also become important tools for the initial identification of newly synthesized compounds, as well as for certain HTS applications and compound profiling efforts. As new types of instruments are developed and as vendors continue to make MS systems that are a modular part of various assay workstations, it is safe to predict that LC–MS will continue to be an integral part of the new drug discovery process.

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