Strategies for dealing with metabolite elucidation in drug discovery and development

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Structural information on metabolites can be a considerable asset for enhancing and streamlining the process of developing new drug candidates. Modern approaches that generate and use metabolite structural information can accelerate the drug discovery and development process by eliminating potentially harmful candidates earlier in the process and improving the safety of new drugs. This review examines the relative merits of current and potential strategies for dealing with metabolite characterization.

Metabolite identification is crucial to the drug discovery process because it can be used to investigate the Phase I metabolites that are likely to be formed in vivo, the differences between species in drug metabolism, the major circulating metabolites of an administered drug, Phase I and Phase II metabolic pathways, pharmacologically active or toxic metabolites and can also help to determine the effects of metabolizing enzyme inhibition and/or induction. The ability to produce this information early in the discovery phase is becoming increasingly important as a basis for judging whether or not a drug candidate merits further development. Metabolite identification enables early identification of potential metabolic liabilities or issues, provides a metabolism perspective that guides the synthetic route with the aim of either blocking or enhancing metabolism to optimize the pharmacokinetic and safety profiles of newly synthesized drug candidates and assists in the prediction of the metabolic pathway(s) of potential drug candidates chosen for development [1–10].

Recent efforts among researchers have focused on developing faster methods for metabolite identification [11–28]. Given the abundance of potential candidate compounds, the characterization of the metabolites generated presents a significant analytical challenge. To achieve a more efficient, practical and reliable method for metabolite identification, in vitro microsomal incubations and sample preparation are important [29]. Robotic systems have been key in the development of sample preparation. The use of such systems [e.g. Genesis workstation (TECAN; http://www.tecan.com)] saves time and provides a high level of confidence and reliability in day-to-day operations [29]. In vitro incubations of drug candidates are often analyzed by liquid chromatography–mass spectrometry (LC–MS). The use of techniques such as nuclear magnetic resonance (NMR) and MS is crucial in metabolite determinations, with NMR spectroscopic techniques the most frequently used to elucidate and confirm metabolite identification in drug metabolism studies. Although LC–NMR is an excellent technique for metabolite determination, LC–MS is often the preferred choice because of the advantages of using MS compared to NMR; MS has a higher sensitivity, requires smaller sample volumes and affords significantly faster sample analysis than NMR. LC–MS–NMR has become commercially available and is used in the late discovery stage to confirm and characterize metabolites. In conjunction with MS, hydrogen–deuterium (H–D) exchange and derivatization methods facilitate structural elucidation and interpretation of tandem mass spectrometry (MS–MS) fragmentation processes [9,30].

In my experience, the best combination for accomplishing rapid, accurate metabolite identification involves a robotic system for
sample preparation and in silico software to predict and find possible metabolites and to predict hypothetical metabolite chemical structure. This can then be combined with the use of LC–MS to determine exact mass measurements (accurate mass) for sample analysis [31], and LC–MS–NMR and online H–D exchange for further metabolite structure confirmation and elucidation.

The relative merits of current and potential strategies for dealing with metabolite characterization in various stages of drug discovery and development are examined in this review and the techniques, tools and approaches suggested for each stage are summarized in Table 1.

### The presynthesis stage of drug discovery

In the presynthesis stage of drug discovery, in silico methods are valuable tools that are used in conjunction with chemical synthesis efforts to select, eliminate or modify the chemical structure of a drug candidate.

In silico screens

The economics of the pharmaceutical industry dictate that large numbers of drug candidates must be examined in drug discovery while simultaneously minimizing development costs and time. With the advent of combinatorial chemistry and high-throughput screening, the number of new candidate structures emerging from the discovery cycle has increased significantly. This has created a demand for earlier and faster determination of the absorption, distribution, metabolism and excretion (ADME) properties of these candidates and it is no surprise that computer software has been developed as a solution to this. Such software packages predict, based on the chemical structures of the drug candidates, potential metabolites and hypothetical metabolite chemical structures and their toxicity profiles and have the ability to screen large numbers of structures even before the chemical synthesis of a drug candidate has started. It has long been recognized that the ability to predict the metabolic fate of a chemical substance and the potential toxicity of either the parent compound or its metabolites is important in new drug design. Commercial computer-based in silico software such as Topkat (Artificial Intelligence Applications Institute; http://www.aiai.ed.ac.uk), Case/Multi-Case (Case Western Reserve University; http://www.cwru.edu), DEREK (LHASA;
University of Leeds; http://www.chem.leeds.ac.uk/luk/derdek), HazardExpert and Pallas (CompuDrug; http://www.compudrug.hu/inside.php), MetaboLynx™ (Micromass; http://www.waters.com) and ACD/MS (Advanced Chemistry Development; http://www.acdlabs.com) has become increasingly popular [2,9,32–36]. From experience of using these software packages, potential pitfalls and disadvantages have been identified for each package. Pallas, MetaboLynx™ and ACD/MS will be discussed in this review.

Pallas software
Pallas uses a comprehensive knowledge base, which consists of a transformation database of reaction rules for animals and humans, to predict the metabolism of exogenous compounds in animals and humans; Pallas can predict the metabolites that could theoretically be formed in Phase I and II biotransformations. The application of Pallas at the presynthesis stage of the drug discovery process can be illustrated using the example of Nimodipine [Nimotop®; Bayer (http://www.bayer-pharmaceuticals.com)] (Figure 1) [9]. Pallas predicted the formation of nine metabolites, but of these only two were observed. Furthermore, three metabolites were identified that had not been predicted by Pallas. This example illustrates a problem with Pallas: because its knowledge base is built on reaction rules that are based on substructures, Pallas overlooks some of the actual or probable metabolites and makes false-positive, or unlikely, predictions. Unusual metabolites, or those that are formed via several intermediate synthetic steps, are also likely to be missed by Pallas. To eliminate these extraneous predictions, the efficient use of Pallas requires an experienced user, or trial and error [2].

MetaboLynx™ software
The majority of companies that produce MS instrumentation also provide metabolite identification software. However, there are potential limitations associated with these packages. Because each software package is designed to work best with the instrument supplied by that company and the specific raw data the instrument generates, each combination of instrument and package must be validated. The software packages and the instruments they are designed to work with are relatively new, and there is much room for development and improvement. For example, MetaboLynx™ can be used to search for expected and unexpected metabolites. When using MetaboLynx™ to search for expected metabolites, the masses of the metabolites are specified and MetaboLynx creates the corresponding extracted ion chromatograms. In the experience of our research group, one problem that was encountered during this step was the number of extra or ‘nonsense’ peaks that were reported, the minimization of which required fine-tuning of the comparison parameters [2]. Another disadvantage associated with this software is that the user must manually enter the expected metabolites. In addition, for the successful implementation of MetaboLynx™, the control sample must be incubated and processed under the same experimental conditions as its corresponding metabolized sample. This disadvantage can be overcome by using an automated robotic liquid handler, which ensures accuracy and consistency in the generating and processing of samples.

ACD/MS
ACD/MS can be used to search for metabolites and to assign potential structures to each metabolite using MS–MS or ion trap (MS3) fragmentation analysis. By generating a percentage for the structure homology of possible metabolites from the data produced by MS analysis, ACD/MS determines the hypothetical chemical structure with the highest probability of being that of the metabolite. It is preferable to have accurate mass data, which significantly improves the confidence level for results, but the program will work without it. Another difficulty encountered is that, in batch mode, the hypothetical modifications must be drawn manually on the structure. In addition, high intensity noise peaks create problems because the presence of these peaks in the spectrum results in ACD/MS returning a low score of spectrum assigned. This is more of a problem for in vivo than in vitro samples. Use of the component
detection algorithm (CODA) enables the user to ‘clean up’ the LC–MS data by reducing the intensity of noise and background data. The use of ACD/MS software in predicting the structure of metabolites, and thus the metabolic pathways, has been recently reported [2].

The value of in silico studies is improved when they are complemented with high-throughput functional test systems. In silico studies cannot replace conventional in vitro or in vivo testing but it is likely that, in conjunction with other techniques, they will streamline the overall discovery process [2]. Information acquired from metabolite identification and characterization is useful as a guide for the efforts of synthetic chemists in the structural modification of new drug candidates. Several modifications can be used to enhance metabolic stability of the drug candidate, including the introduction of halogen elements (e.g. F, Cl and Br) to increase the electronegativity and lipophilicity, the addition of a bulky group to inhibit access to the moiety that was metabolized and cyclization to minimize metabolism of the drug.

Early drug discovery and screening

In the stage of the drug discovery process following synthesis, compounds are made available for screening. At this point, in silico methods are still useful, in conjunction with LC–MS–MS, for directing synthetic efforts to block or enhance metabolism, for identifying minor and major metabolites (e.g. dealkylations and conjugations such as glucuronide) and for predicting metabolites that are likely to be formed in vivo.

LC–MS

For nearly every ionization method, a procedure has been developed to perform metabolite identification. Examples of these procedures include electron impact (EI), chemical ionization (CI), atmospheric pressure chemical ionization (APCI), fast atom bombardment (FAB), thermospray, gas chromatography–mass spectrometry (GC–MS) and electrospray ionization (ESI) [37–39]. Currently, the majority of the analytical characterization of metabolites is performed using LC coupled with atmospheric pressure ionization–MS using MS² or MS–MS. The use of MS² enables the relatively rapid construction of fragmentation maps and a higher degree of specificity. The advantages of using quadrupole time-of-flight (QTOF)–MS for metabolite identification compared to MS² or triple quadrupole mass spectrometers include faster mass spectral acquisition speed with high full-scan sensitivity, enhanced mass resolution and accurate mass measurement capabilities that allow for the determination of elemental composition. Exact mass measurement is highly useful for the confirmation of elemental composition and is a valuable tool for solving structure elucidation problems.

Increasing sample complexity, sample volume restrictions and throughput requirements necessitate that the maximum amount of useful information is extracted from a single experiment. Data-dependent scans performed on QTOF or MS³ are excellent tools that are used to acquire significant volumes of information. Data directed analysis (DDA), or data-dependent scans, enable intelligent MS and MS–MS acquisitions to be performed automatically on multiple co-eluting components. DDA uses the inherent high resolution, exact mass measurement capability and full mass range to make decisions about which ions to select for MS–MS. However, one shortcoming of this technique is that, as a result of limited sensitivity, data-dependent scans frequently fail to identify minor metabolites.

Use of LC–MS to identify and characterize metabolites

LC–MS is used in various strategies for identifying compounds and/or their metabolites either to confirm the structure of a known compound or to identify unknown metabolites of drug candidates. For example, when searching for metabolites in full-scan mass spectra the procedure typically followed is to identify the most intense ion with singly-charged ions, or doubly-charged ions at 1/2 m/z, adducts, multiply-charged ions and/or dimers in the full-scan spectrum. The nitrogen (N) rule states that if a drug or compound contains no, or an even number of, N atoms then the molecular ion of the compound will have an even mass number; an odd number of nitrogen atoms causes the molecular ion to have an odd mass number. Application of this rule to the molecular ion determines if the unknown has an even or odd number of N atoms. Next, the isotopic peak of the molecular ions is checked to confirm patterns, but the possibility of interference from other ions must be considered. Application of the double-bond-equivalents (DBE) rule determines or confirms the total number of rings plus double bonds present in a compound containing carbon (C), hydrogen (H), N, oxygen (O) and other elements that have the same valence. Using CxHyNzOn as an example,

\[
\text{DBE} = y + 2x + z/2 + 1
\]

where \(x\) = number of C atoms, \(y\) = number of H atoms, and \(z\) = number of N atoms

Accurate mass measurement is then performed to confirm and/or determine possible molecular formulae. Alternatively, LC–MS can be used to compare product ion MS–MS or MS³ spectra of the parent with metabolites. Once molecular ions for possible metabolites are identified, they can be subjected to analysis by MS–MS or MS² and the product ion MS–MS or MS³ spectra of the parent
Figure 2. Representative TOF-MS-MS spectra [M+H]⁺ m/z 272 of (a) nonmetabolized dextromethorphan and [M+H]⁺ m/z 258 and (b) dextromethorphan-metabolite following incubation of dextromethorphan with rat liver microsomes. Each arrow indicates a possible site of fragmentation, with the corresponding ion. Abbreviation: TOF-MS-MS, time of flight–mass spectrometry–mass spectrometry.
compound can subsequently be compared with the corresponding spectra of the metabolite. Using the known structure of the parent drug and its corresponding fragmentation pattern as a reference facilitates the elucidation of metabolite structure. The specific fragment ion that showed a shift in its $m/z$ helps to identify the site of the modification of the molecule. For example, the time-of-flight (TOF)–MS–MS fragmentation for dextromethorphan (Figure 2) shows a product-ion spectrum of $m/z$ 272 and fragment ions of $m/z$ 213, 198, 171, 147, 121 and 91. QTOF–MS–MS fragmentation for the metabolite dextrorphan (Figure 2) shows a product-ion spectrum of $m/z$ 258 and fragment ions of $m/z$ 199, 157, 133 and 107, which suggests that dextromethorphan has undergone O-dealkylation. The specificity of product ion MS–MS or MS$^{n}$ spectra facilitates the elucidation of proposed metabolite structures. The proposed metabolic pathways of dextromethorphan in hepatic microsomal incubations are illustrated in Figure 3.

**Late drug discovery and/or candidate selection**

As drug candidates reach the next phase, late drug discovery and/or candidate selection, LC–MS–MS, QTOF (high resolution and exact mass measurement), MS$^{3}$ and H–D exchange are used to determine metabolic differences between species and to identify potential pharmacologically active or toxic metabolites.

Exact mass analysis is used to confirm elemental composition of metabolites

The exact mass (accurate mass) shift between a drug candidate and its metabolites can be used to predict the elemental composition of those metabolites. The elemental composition of the metabolites is generated from the full-scan mass-spectra, and hence the molecular formulæ of the unknown metabolites can be deduced. Subsequently, the molecular formulæ can be used to identify the metabolites and, based on additional MS data and tools, a structure can be proposed. Thus, confirmation of a metabolite can be accomplished by establishing agreement between its proposed structure and its accurate mass.

Most mass analysis relies on ‘nominal’ mass accuracy (i.e. to within 1 Da). However, an increasing number of applications are based on much more accurate mass measurement. Exact mass measurements at high resolution of a QTOF instrument [exceeding $m/\Delta m = 8000$, where $m$ is the $m/z$ value of the peak and $\Delta m$ is the width ($m/z$) of a triangular peak across the base] decrease the probability of interference occurring from non-compound related ion peaks. To address the whole mass range and to avoid isobaric interferences, it is helpful to carefully select the mass calibration reference (isobaric interferences are extraneous ions resulting from, for example, the mobile phase and the mass calibration solution; these can be avoided by using complementary mass calibration). Electrospray orthogonal TOF mass spectrometers are capable of generating mass spectral data of sufficient accuracy and precision to establish the elemental composition of an analyte. Exact mass analysis of several drugs and their metabolites was performed using TOF–MS at resolutions in the region of 8000 full width half maxima (FWHM) with MetaboLynx™ 3.5 [2]. For selected drugs, we found a good correlation between calculated and experimentally observed masses. All measured values were accurate to less than or equal to 0.0010 Da and the standard deviation was within 0.0015 Da, which enabled good prediction and confirmation of empirical formulæ [2]. By decreasing the probability of interference occurring from non-compound related ion peaks, accurate mass measurement leads to increased confidence in the ability to identify metabolites in drug discovery studies. Accurate
mass measurement can also be conducted on fragment ions to confirm proposed fragmentation mechanisms.

Online H–D-LC-MS and derivatization
H–D exchange occurs in solution when there are exchangeable (labile) hydrogen atoms present in a molecule. One advantage of the H–D exchange method is that, when used in conjunction with LC–MS, it facilitates the estimation of the number of labile hydrogen atoms in groups such as –OH, –SH, –NH, –NH₂ and –COOH. Knowledge of the number of labile H atoms in a molecule is useful for comparing metabolite structure with that of the parent drug to determine the presence or absence of these functional groups [9,40–46]. In addition, H–D exchange

Figure 4. Representative TOF-MS-MS spectra of Nimodipine-metabolite in (a) D₂O m/z 408 and (b) H₂O m/z 405 following incubation of Nimodipine [Nimotop® (Bayer; http://www.bayer-pharmaceuticals.com)] with human liver microsomes. Each arrow indicates a possible site of fragmentation, with the corresponding ion. Abbreviation: TOF-MS-MS, time of flight–mass spectrometry–mass spectrometry.
experiments facilitate structural elucidation and interpretation of MS–MS fragmentation processes. A method for metabolite identification in drug discovery and development that uses online H–D exchange and a tandem QTOF mass spectrometer coupled with liquid chromatography (LC–QTOF–MS) has been recently developed [9]. This method appears to be highly effective for identification of metabolites produced by dehydrogenation, oxidation, glucuronidation, and dealkylation. Using this approach, N- or S-oxide formation and mono-hydroxylation can be differentiated; furthermore, conjugation, for example, formation of quaternary amine glucuronide versus primary or secondary glucuronide, can be easily identified using this technique. The generic method has proved to be simple, easy, fast, sensitive, robust and reliable and enhances throughput, which in turn facilitates the ability to rapidly provide characterization of metabolites in vitro or in vivo. In addition, online H–D–LC–MS is suitable for low resolution instruments and, taken together with the particular benefits of using QTOF (e.g. higher resolution), these advantages make this method a valuable tool for structure elucidation.

Nimodipine can be used as an example of the use of online H–D–LC–MS. The proposed TOF–MS–MS fragmentation for metabolite M-4 of Nimodipine in D$_2$O and H$_2$O is shown in Figure 4a and 4b, respectively. The full-scan mass spectrum of metabolite M-4 of Nimodipine revealed a protonated molecular ion [M$_{H}$$^+$H$^+$] at m/z 405, 14 atomic mass units (amu) lower than Nimodipine, which suggested that metabolite M-4 was a product of the cleavage of Nimodipine. The production spectrum of [M$_{H}$$^+$H$^+$] at m/z 405 showed fragment ions of m/z 361, 343, 317, and 301 (Figure 4b). When H$_2$O was replaced with D$_2$O in the mobile phase, the full-scan mass spectrum of
Nimodipine revealed a molecular ion \([M+D]^+\) at \(m/z\) 408, three amu higher than \([M+H]^+\) M-4, which indicates the presence of two labile hydrogen atoms in M-4. The product-ion spectrum of \(m/z\) 408 showed fragment ions of \(m/z\) 363, 344, 319, and 302 (Figure 4a). This suggests that M-4 derives from the cleavage of Nimodipine. The proposed metabolic pathways of Nimodipine in hepatic microsomal incubations in \(H_2O\) and \(D_2O\) are shown in Figure 5.

Derivatization of a metabolite can result in stabilization of unstable metabolites, improvement of the chromatographic properties of polar compounds and reduction in the volatility of volatile metabolites. In addition, derivatization can be useful in the characterization of chirality and the site of metabolism. Numerous methods of derivatization have been reported that can be used to identify the majority of the chemical functional groups [47]. One limitation of these techniques is the time-consuming sample preparation. To derivatize metabolites in a sample, it is necessary to either isolate the specific metabolite before derivatization, or derivatize the entire sample, which will create more complex ions in MS. Because online H–D–LC–MS is a much faster technique and requires less sample preparation, and LC–MS–NMR has become more widely available and more sensitive, derivatization methods have fallen out of favor.

Preclinical and clinical development

During the fourth stage of the drug discovery process discussed in this review, LC–MS–MS, QTOF (high resolution and exact mass measurement), MS3, and H–D exchange are the major tools that are used for analysis of metabolites. Once the candidates reach this stage, most become available in radiolabeled form and, therefore, radioactivity detectors can be used for mass balance studies. Some of the tools used in previous phases are still used in this stage. NMR is used as an additional tool to determine the percentage of metabolite formed in vitro or in vivo, the definitive structure of the candidate for the synthesis of metabolites for toxicology testing and the comparison of human pathways and drug-drug interactions.

LC–MS–NMR

NMR spectroscopy is one of the most commonly used techniques for the determination of metabolite structure. Technical advances in NMR, including the introduction of modern high-field strengths (500, 600 and 800 MHz NMR spectrometers) have increased the sensitivity of NMR spectroscopy. The most common nuclei detected by NMR spectroscopy in the analysis of drug metabolites are \(^1H\), \(^19F\), \(^13C\), \(^31P\) and \(^15N\). NMR sample concentrations must be between 1 \(\mu\)g and 10 mg; sample analysis can take as little as 5 min or as long as 1 h. There are several operational modes that can be employed for LC–MS–NMR, the conditions of which can include isocratic or gradient elution, continuous-flow, stop-flow, time-sliced stop-flow, peak collection into capillary loops for post-chromatographic analysis and automatic detection of chromatographic peaks with triggered NMR acquisition. Depending on the sample concentration and chromatographic resolution required, any of these techniques could be employed [48].

The online H–D–LC–MS method has the ability to rapidly characterize metabolites [9]. However, NMR is still required to characterize the regiochemistry of aromatic oxidation, to determine the site of aliphatic oxidation where fragmentation pathways are unavailable or inconclusive and to locate functional groups such as OH, epoxide and sulfate by comparing NMR spectra of the parent with those of the metabolite. In the identification of unknown metabolites, NMR and MS are clearly complementary and analytical data from both methods are often required to definitively confirm a metabolite structure. LC–MS–NMR enables the acquisition of high quality data and has enormous benefits because of the dual nature of the information collected from the same analytical run. These systems are in the midst of an evolutionary phase that will lead to greater sensitivity and the ability to improve efficiency in terms of the chromatographic properties; as this promise is realized, LC–MS–NMR will become a mainstream approach.

Online LC–ARC coupled with MS

The use of radioactively labeled drugs is important in the study of many aspects of drug metabolism including, ADME, bioavailability, biotransformation, metabolite identification and other pharmacokinetic studies. The radioactive isotopes \(^14C\) or tritium (\(^3H\)) are typically used for the labeling of a given drug. HPLC separation works well with radioactive labeling, enabling high resolution, quantitative detection of unknown metabolites and real-time monitoring by connecting the HPLC-radioactivity detector outlet to the mass spectrometer. These detector interfaces are useful for generating data for the structural elucidation of metabolites and of biotransformation pathways for an administered drug. It has been reported that a microplate scintillation counter combined with capillary LC can be used to enhance sensitivity through eluent fractionation and subsequent offline counting [49]. The limitations with this method are that the sample must be completely dry before counting, any volatile compounds will probably be lost and there is the potential for apolar compounds to adsorb onto the surface of the plate. Accelerator mass spectrometry (AMS) has been applied to the detection of \(^14C\)-labeled triazine metabolites in urine. The limitations associated
with these techniques include time-consuming sample preparation, high analysis costs and the inability to elucidate metabolite structure.

A novel detection method combining online LC–ARC (liquid-chromatography–accurate radioisotope counting; advanced stop flow controller) coupled with a radioactivity detector and mass spectrometer was developed for metabolite identification in drug discovery and development [50]. One of the major benefits of using this method is that the system enhances the sensitivity of radioisotope measurement. Another advantage associated with using this system is the simple interface with the mass spectrometer, which facilitates acquisition of online mass spectrometric data. The use of LC–ARC dramatically improved the sensitivity for 14C peaks by up to 20-fold compared with conventional flow-through detection methods. Furthermore, no fraction collector or time-consuming sample preparation is needed. The system gives accurate column recovery, quantification of low-level radioactivity and high resolution is maintained throughout the run. The overall results suggest that the combination of LC–ARC with radioactivity detection and MS has great potential as a powerful tool for improving the sensitivity of radioisotope measurement in metabolite identification studies during drug discovery and development. Furthermore, the study illustrates the impressive progress that has been made in the technology of radioisotope counting in drug metabolism using LC–ARC [50].

Conclusions
This manuscript describes the author’s opinion on conducting metabolite identification in drug discovery and development. Modern MS and NMR instrumentation can greatly assist the analyst in providing high-quality data in a more rapid and time-efficient manner. Continued improvement of the databases on metabolic pathways could be combined with new and improved high-throughput testing systems to streamline the process. The ideal metabolite scientist should have a good working knowledge of the four pillars of drug metabolite characterization: MS, NMR, chemistry and metabolism. Cross-training and sharing resources in these four areas will accelerate progress toward faster metabolite identification of drug candidates. In silico studies have clear benefits; however, they must be used carefully, and are most effective in combination when used with other techniques and technologies. Accurate mass measurement enables increased confidence in the ability to identify metabolites in drug discovery studies by decreasing the probability of interference occurring from non-compound-related ion peaks. Furthermore, when used in combination with other data, accurate mass measurement is useful for elucidating metabolite structure. The dual data and complementary nature of NMR and MS is valuable in elucidating definite metabolite structures much more rapidly, as a consequence of improvements in the sensitivity and efficiency of these techniques. Online H-D-LC-MS and LC-MS-NMR have become more widely available and sensitive, making them the tools of choice for metabolite confirmation. It is the opinion of the author that improvement in the drug discovery process will be facilitated most by learning how and when to use each of these tools to achieve the maximum benefit, and by increasing cooperation and dialogue among the disciplines involved in the entire process.

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