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Review

A review of LC–MS techniques and high-throughput approaches used to investigate drug metabolism by cytochrome P450s☆

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ABSTRACT

One of the major challenges facing the pharmaceutical industry today is finding new ways to increase productivity, decrease costs whilst still ultimately developing new therapies that enhance human health. To help address these challenges the utilisation of analytical technologies and high-throughput automated platforms has been employed; in order to perform more experiments in a shorter time frame with increased data quality. One of the main *in vitro* techniques to assess new chemical entities in a discovery setting has been the use of recombinant liver enzymes, microsomes and hepatocytes. These techniques can help predict *in vivo* metabolism, clearance and potential drug–drug interactions of these new compounds by cytochrome P450s (the major drug metabolising enzymes). This *in vitro* methodology has been totally transformed in recent times by the use of automated liquid handling and HPLC tandem mass spectrometry detection techniques (LC–MS/MS). This review aims looking at recent advances in the methodology used to investigate drug metabolism by cytochrome P450s; including an up to date summary of high-throughput platforms including the use of automation and LC–MS/MS to facilitate greater throughput, chromatographic resolution and data quality.

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1. Introduction

The "drug metabolizing enzymes" (DMEs) are a diverse group of proteins that are responsible for metabolizing a vast array of xenobiotic compounds including drugs, environmental pollutants, and endogenous steroids and prostaglandins (http://www.invitrogen.com/etc/medialib/en/filelibrary/pdf.Par.58399.File.dat/O-12758DMEGuide_Intro.pdf). These enzymes can be separated into two groups, namely oxidative and conjugative. Oxidative enzymes, largely contribute to so-called Phase I metabolism, which include cytochrome P450s (CYPs) and flavin monooxygenases (FMOs), which catalyse the introduction of a reactive oxygen atom into a lipophilic compound. If the metabolites of Phase I reactions are sufficiently polar, they may be readily excreted at this point. Phase II reactions involve the addition of an endogenous substrate with either the newly incorporated functional group derived following Phase I metabolism or to that of a pre-existing functional group. This serves to increase polarity further facilitating excretion from the body. The cytochrome P450 family of enzymes have to date received the greatest attention owing to their role in the metabolism of the majority of drugs in humans. In this regard, CYPs 1A2, 2C9, 2C19, 2D6 and 3A4 together have been reported to be the major route of metabolism for almost 75% of the 200 most commonly prescribed drugs [1].

A requisite of any drug entering the marketplace is an understanding of its potential to affect one or more of these CYPs and elicit a drug–drug interaction (DDI); where the maximum plasma concentration of any one or two drugs co-administered together increase by more than 2-fold. For certain drugs this results in toxic concentration in the circulation which ultimately results in hospital admissions [2] and if reactions are significantly adverse, potentially drug withdrawal from the marketplace [3]. Given that the simultaneous co-administration of multiple drugs is common place increases the likelihood of clinically significant interaction, in particular in patients receiving multiple therapies for one disease (e.g. HIV infection) or treatment for several diseases concurrently [4,5]. The pharmaceutical industry has had to invest significant resources to understand these risks, since the progression of compounds with potential DDI liabilities will lead to labelling restrictions as well as having a potential competitive disadvantage.

Screening for unfavourable drug-like properties, such as interactions with cytochrome P450s early on in the drug discovery process can potentially avoid the excess cost of developing unfavourable drug candidates. A screening strategy is designed and implemented to provide the greatest amount of quality data to inform project decisions early in discovery, but at the same time minimise overall costs [6]. In order to meet this challenge, a significant amount of effort is concentrated towards implementation of targeted *in vitro* assays at the different stages of the drug discovery process. These *in vitro* assays are executed with sample preparation in miniaturized micro-plate formats using advanced automated liquid handling technologies; producing high numbers of experiments and samples [7]. These samples are then analysed using a number of high-throughput analytical platforms based around rapid high-performance liquid chromatography (HPLC) and tandem mass spectrometric detection (Table 1). These analytical platforms provide increased efficiency in running these screens with robust, multi-analyte quantitation capability.

This article will summarise the mass spectrometric technology, HPLC configurations, high-throughput platforms and automation utilised in the field of drug metabolism by cytochrome P450s. A summary of most recent applications of these technologies will be described in the various *in vitro* studies carried out in the pharmaceutical discovery arena.

2. Mass analysers for liquid chromatography

Due to the high specificity, speed and selectivity offered by HPLC–MS/MS, this approach has long been adopted in the pharmaceutical industry to assess certain properties of drug molecules, such as metabolic stability. Given the large number of mass spectrometer types available and that their application differs between laboratories, means the approaches used for metabolism studies will inherently differ from laboratory to laboratory. As such the data quality and reliability of the results strongly depend upon which instrumentation is optimal for the task [8]. A brief overview of the mass spectrometers used in the assessment of new chemical entities (NCEs) and their associated metabolites will be covered.

2.1. Quadrupole mass analysers

Single-Stage quadrupole mass spectrometers (SSQMS) as well as triple-stage quadrupole mass spectrometers (TSQMS) are commonly used by the pharmaceutical industry for both qualitative and quantitative studies [9]. Whilst quadrupole mass analysers have the ability to operate in both negative and positive ion modes, specific advantages of SSQMS instruments include low cost and their relatively small size, whilst TSQMS instruments have greater discrimination against chemical background resulting in real gains in selectivity and sensitivity. In TSQMS, the Q1 mass analyser filters the desired ions such that they are fragmented by Ar or N_2 within Q2, and their fragment ions are subsequently scanned by Q3 before reaching the mass detector. Consequently, given that TSQMS acquires much richer, higher value datasets than SSQMS and in selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) modes would suggest it to be the instrument-of-choice in routine and high-throughput quantitative bioanalysis. However detection sensitivity decreases dramatically when wide mass range is analysed in a scanning mode; which can be a limitation in its application for screening of '*unknown*' drug metabolites.

2.2. Ion-trap mass analysers

Like quadrupole instruments, ion traps are relatively inexpensive and compatible with a wide range of systems. Ions generated are focused towards the centre of the trap allowing measurement of all ions retained in the trapping step. Consequently, sensitivity losses during the full-scan mode are avoidable. Whilst TSQMS retains sensitivity advantage for quantification when operated in SRM mode, ion-trap instruments provide more sensitivity for structure elucidation than TSQMS. This is due to the fact that ion traps can obtain richer mass spectra, with more efficient trapping and scanning of ions; this MS mode can be more structurally informative when compared to triple quadrupole or Quadrupole time-of-flight (Q-TOF)-mass spectrometers. The ion-trap analysers can be used for quantification in full-scan mode, with little difference between in sensitivity in SIM or MRM modes. When operated in a full-scan mode, the sensitivity gains, ability to measure a wide mass range and acquisition of full-scan data can make these instruments ideal for screening-type applications in which qualitative information is paramount (such as metabolite identification studies) [10]. With the advent of increased computer power and data storage, increased capture of full-scan data can enable mining of this qualitative information at a later date, when more is known about a compounds metabolism. These instruments have traditionally shown increased variability at the limits of detection due to the slow ion accumulation time. This coupled with the relatively slow data acquisition rate, limits their use in high-speed quantitative LC–MS applications, such as fast UPLC analyses. In recent years, linear ion traps have been developed. Their configuration is similar to quadrupoles, with a barrier at the end to prevent ions exiting. Ions