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**Sai Laxmi Kesana**
College of Technology, Osmania University, Hyderabad, Telangana, India

**Dodle Jaya Prakash**
College of Technology, Osmania University, Hyderabad, Telangana, India

**Borra Pooja**
College of Medicine, Davao Medical School Foundation Davao, Philippines

**PC Sastry**
Department of Mechanical Engineering, The Institution of Engineers, Baroda, Gujarat, India

**Correspondence**
**Sai Laxmi Kesana**
College of Technology, Osmania University, Hyderabad, Telangana, India

# Importance of *in vitro* met id studies

**Sai Laxmi Kesana, Dodle Jaya Prakash, Borra Pooja and PC Sastry**

**Abstract**
Metabolism related liabilities continue to be of major concern in clinical development of a drug candidate. A complete understanding of the relationship between *in vitro and in vivo* metabolism is important. Metabolite profiling and drug phenotypic studies using specific CYP enzymes would selectively demonstrate the metabolic pathway and the interactions of the drug without the usage of animal models. In many cases *in vitro* studies, owing to their inexpensiveness and ease of carrying, serve for adequate screening mechanism that can rule out the importance of the *in vivo* metabolic studies and make *in vivo* testing reasonably less and even unnecessary in the early screening, in addition the clinical relevance of the results obtained using human microsomes is comparable and yet times give the complete understanding about the compounds behaviour very aptly.

**Keywords:** Metabolism, major concern

## Introduction
Mucoadhesives are synthetic or natural polymers that interact with the mucus layer covering the mucosal epithelial surface and they are the main molecules constituting a major part of mucus [1]. The concept of mucoadhesives has attracted the interest of many investigators and the possibility that these polymers can be used to overcome physiological barriers in long-term drug delivery is now receiving significant attention.

Extensive research Metabolism related liabilities continue to be of major concern for drug candidates in clinical development. The safety concerns arise from the bio activation of the parent compound to a reactive metabolite which brings on biological modification by covalent binding to tissues, redox reactions, formation of toxicants with systemic exposure and the adverse effects. If such information is available at an early stage during the drug discovery, then the chemical structure of the compound can be modified to reduce the adverse reactions of the drug.

The high throughput screening has changed the way drugs are discovered today compared with 20 years ago when fewer compounds were tested in animal or organ models. However in past, drugs have been developed successfully without the evaluation of metabolic routes of elimination during the later phases of drug development or were not explored at all. Today, it is difficult to release a drug without the knowledge of how it is metabolized or how it could influence, or be influenced by the drugs being taken along with it. Therefore, researchers are encouraged to conduct the metabolic studies prior to commencement of phase trials. Hence, considering the above reasons it is most appropriate to conduct metabolism studies and determine the way forth.

Metabolic processes influence many other parameters that are relevant viz., bioavailability, systemic clearance and toxicity; issues relating to drug metabolism are important in the selection of viable drug candidates. Substantial emphasis was placed in this regard on the advancement of knowledge and the development of new technologies in the areas of absorption, distribution, metabolism and excretion (ADME) and aid in the identification of compounds which can survive as drug molecules in the early stages of drug discovery, rather than the determination of the fate of the drug during late clinical development.

Recent efforts to improve the quality of later trials and to ensure about the safety of metabolites the MIST- Metabolite Identification in Safety Testing is important. The United States Food and Drug Administration have prompted the pharmaceutical industry to develop alternative strategies for assessing human exposure to metabolites earlier in the development process. As an alternative to the widespread adoption of animal models in the assessment of oral exposure -Drug-metabolism screening assays with the measurement of parent-compound disappearance, the hepatic CYP activity in liver microsomes are an essential way out of

screening. The identification of bio activation pathways which might produce toxicity and the prediction of interactions that might lead to alterations in the pharmacokinetic profiles continue to be of major challenge in drug discovery. It is believed that technological advancements adopted recently will
allow the researcher- streamline the selected lead candidates, and develop higher quality drugs. Evidence has emerged that these investments have paid off in helping to reduce the number of compounds with unacceptable pharmacokinetic properties. However much progress is still to be made in reducing attrition of other biotransformation-related liabilities [1 -5].

**Importance of metabolite studies and the process**

The main objectives of conducting drug metabolism studies are

a. To identify and characterize the major metabolites of the test compound and specific enzymes responsible for its metabolism. To evaluate the impact of the metabolites on safety and efficacy of the drug and to utilize the information and maximize its intellectual property.
b. During drug development, the mandatory investigation of a drug to assess the clinical safety apart from its efficacy are done, using the *in vitro* studies and an early understanding about the most common metabolites, the behavior of the drug inside the system and benefits of the test compound over the several pharmacologically active agents are drawn.
c. The mechanistic pathway and the extent of metabolism in various species is understood
d. The major metabolites of the test drug and specific enzymes responsible for its metabolism are identified whereby the safety of the compound as drug is evaluated.
e. Aid in preclinical screening of the drug by observing-metabolites and elimination routes without utilization of animals
f. The in *vitro* results, provide a reliable extrapolation to *in vivo* results for various species without effecting the morbidity, mortality and ethical principles.
g. Opens out the available strategies that can be used to uncover metabolites- First in, *in vitro* followed by *in vivo* work up, under a pre-clinical set up, thereby providing the first hand information about the probable metabolites, thus reducing the liabilities involved in the modification of structural features for toxic drug-like molecules.

With these objectives in mind, an understanding of the metabolic profile of the drug would be useful prior to the initiation of phase I studies, and are especially important before phase 3 trials, when a broader population will be studied.

Recent efforts to improve the quality of later trials ensure that the safety of metabolites is adequately tested - United States Food and Drug Administration, (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079266.pdf) have prompted the pharmaceutical industry to develop alternative strategies for assessing human exposure to metabolites earlier in the development process. The introduction of new analytical techniques, like liquid chromatography/mass spectrometry (LC/MS) has made it possible to characterize the chemical properties, metabolic stability and metabolic fate of a large number of compounds before being carried out via studies with a radio labeled version of the drug during late clinical development.

The outcome of the metabolite identification studies are –

i. Detection of metabolites that could be pharmacologically active and contribute to the efficacy of a new chemical entity (NCE), and
ii. Elimination of compounds that form reactive intermediates or toxic metabolites with serious adverse effects [6-9].

**Drug withdrawals from the market**

A number of drugs have been withdrawn from the market and or severely restricted in their use because of unexpected toxicities. According to the FDA, a "drug is removed from the market when its risks outweigh its benefits. A drug is usually taken off the market because of safety issues with the drug that cannot be corrected, such as when it is discovered that the drug can cause serious side effects that were not known at the time of approval." The FDA also takes into account the number of people taking a drug being considered for removal so as not to harm those patients. Globally around 638 drugs have been abandoned and withdrawn from the market in between the years 1985-2010. Some of them were used in only certain populations after being observed of their selective metabolism. A small list of 10 drugs are shown in the below Table.

**Table 1:** Details of some of the withdrawn drugs in the world due to toxic effects

| Name of Drug | Year | Place | Reason for Withdrawal |
|---|---|---|---|
| Cisapride | 2000 | US | Cardiac arrythymia |
| Alsetron | 2000 | US | Serious GIT adverse effects, Constipation |
| Temazepam | 1999 | Norway | Death, whereas used in US |
| Trovafloxacin, Altrafloxacin, Troglitazones | 2000 | EU & US | Liver damage |
| Rosiglitazones | 2010 | EU | Drug toxicity |
| Rapacuronium | 2001 | Multiple markets | Bronchospasm |
| Benzitramide, Co proximal | 2004 | Norway | Dose disorders- metabolism related adverse events |

Metabolite identification has become important for drug safety, where chemically reactive and pharmacologically active metabolites. Metabolites if formed at greater than 10 percent of parent's systemic exposure at steady state, there is a definite need to characterize and evaluate nonclinical safety similar to the parent in the drug safety assessment.

Results obtained from these identification studies are used to refine drug candidates through modifying moieties where major metabolic reactions take place and assess the safety and availability of the drug in the biological system.

The availability of specific CYP enzymes has made it possible to "phenotype" Test compounds with respect to their

relative levels and their metabolic capabilities. The various CYP enzymes differ in their substrate specificity and the metabolism of the probe substrate. Thus screening of compounds using the human CYP (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) would enable the researcher understand clear drug metabolic pathway. These processes have come in place after the introduction of new analytical techniques, especially HR-LC/MS which can characterize the metabolites, metabolic stability and metabolic fate of a large number of drugs while screening for further development.

As part of the MIST and ICH Guidelines on drug safety recent efforts were made to improve the quality of later trials to ensure the safety of metabolites is adequately tested, United States Food and Drug Administration have prompted the pharmaceutical industry to develop alternative strategies for assessing human exposure to metabolites earlier in the development process. The success of this approach depends on the comprehensive detection and identification of relevant metabolites using *in vitro* sample [10-15].

**Tools to characterize drug metabolites**

LC/MS has become widely used tool in the identification, structure characterization and quantitative analysis of drug and metabolites. Today MS methodologies are enabled for fast, sensitive, and accurate metabolite identification. These instruments were utilized in cases where determination of empirical formulae of metabolites or their fragments were required. New improved HR-MS instruments and data processing techniques have been developed for metabolite identification. These configurations are useful in metabolite identification with a highly resolved and accurate MS/MS spectra achieved through ion fragmentation. The coupling of liquid chromatography (LC) to MS (LC-MS) facilitates metabolite identification and Quantitation by reducing sample complexity and allowing metabolite separation prior to detection.

In the analysis, data acquisition is performed with the $MS^E$ scan function. Metabolite identification software screens all the ion chromatograms of the expected metabolites, and reveals possible metabolic reactions and related changes in masses of the tested drugs. For a large number of metabolites, a computational frameworks are developed, which can reduce the number of putative identifications. The framework primarily focuses on untargeted endogenous metabolomic studies and uses *m/z* values and MS/MS spectra while Elemental compositions are used for ion annotation. Modern HR-MS instruments provide ion measurements with high-resolution >10,000 at full-width at half-maximum, and accurate mass with less than 5 ppm deviation capabilities. This enables collection of data that can distinguish drug metabolites from most of endogenous components and can give elemental compositions of metabolite ions and their fragments.

Metabolites are generated *in vitro* with the use of animal and human recombinant expressed enzymes, liver and other tissue fraction homogenates e.g. liver microsomes or S9 with necessary cofactors included, with an incubation followed by characterization using LC-MS/MS for the drug and its conjugates. These techniques can be initially checked using model compounds and later applied for the investigational and research molecules. *In vitro* animal models are used to predict the metabolic fate of drugs in animals while comparing with the humans and the clinical relevance of the results obtained is noted. This step is significant as using animal models it is difficult to assess and yet times not predictable which can sometimes be predicted from *in vitro* studies. CYP *in vitro* studies using hepatic microsomes incubation with test compounds, cofactors determine metabolites of compounds and provides clear picture of the probable nature of the drug inside the biological systems.

This work up is designed to have data during drug development using *in vitro* studies. The *in vitro* studies being conducted give an early understanding about the most common metabolites, the behavior of the drug inside the system and benefits over the several pharmacologically similar agents. Metabolites are observed *in vitro* with the use of animal and human recombinant expressed enzymes, liver and other tissue fraction homogenates e.g. liver microsomes or S9 with necessary cofactors included, with an incubation followed by characterization using LC-MS/MS for the drug and its conjugates.

Results obtained from these identification studies are used to refine drug candidates through modifying moieties where major metabolic reactions take place, then assess the safety and efficacy of the drug [16-20].

**Comprehensive profiling of metabolites and future perspective [21-28]**

Metabolite profiling is a main drive in the drug discovery process to optimize pharmacokinetic properties and to deliver safer drugs. The untargeted, metabolic profiling using high resolution analytical platform can be worked up for research compounds. Simultaneous extraction and separation technique in combination with high resolution accurate mass spectrometric detector aids in the identification of the parent and its metabolites based on the theoretical interpretation in support to the MS/MS. The 60 min incubation in human liver microsomes suggests the formation of major metabolite which gives a preliminary data in the respective microsomes desired. The data obtained from various species like monkey, dog, rat and mice suggest the formation of metabolites and the selection of the desired animal model. These are confirmed by MS/MS and Accurate mass analysis using the analytical tool, LC-Q-TOF Mass spectrometry. The drug's metabolism in mice and rat in comparison to the human, monkey and dog liver microsomes usually would give sufficient in site as numerous reports have documented the difference in the metabolic fate of drugs between animal species.

The observed differences and coherences between rodents and non-rodents would give enough confidence to consider the right model while working on specific pathways in future. For the test compound if the major metabolic pathways observed were found to be by the oxidation, then the compound would be a safer one. Later for confirmation the *in vitro* study shall be investigated by *in vivo* model and checked for the metabolites from the experimental model and commented.

Phenotype Studies convey the action of the specific CYP enzymes which give lead about the half life of the Test compound, demonstrating the molecule's metabolism by the selective microsomal enzymes and how the Test compound is influenced by a group of enzymes at different levels during its metabolism.

The integration of biotransformation studies into a drug-discovery workflow fulfills few important objectives the first is a concerted effort between drug metabolism and medicinal chemistry groups to identify metabolism-related liabilities of compounds in a timely manner, and then use of this information effectively in managing the evolution of a lead

series, while the second is to continually evolve with the practices in accordance with changing regulatory requirements, technological improvements and knowledge, that lies between drug metabolism and toxicology. This dynamic paradigm is expected to assist in the advancement of drug candidates with a lowered metabolism-related liabilities and commensurately improved chances for success in the clinic. Ultimately, it is hoped that these changes will expedite the delivery of effective and safe new medicines to the patients who need them.

Biotransformation studies play an important role in the identification of several compounds like, the formation of a reactive metabolite, or the covalent modification and irreversible inhibition of the enzyme. Such behavior can usually be attributed to chemical structures that also have a proclivity toward bio activation and the formation of protein conjugates. However, the presence of GSH may fail to prevent the onset of irreversible inhibition, since binding may occur before the substrate has a chance to leave the active site. A separate set of experiments determine whether the compound of interest may carry out the liable mechanism-based inhibition or not.

A complete understanding of the relationship between *in vitro and in vivo* metabolism is important.where *in vitro* experiments conducted at concentrations similar to the relevant concentration of *in vivo*,are essential as in real studies, different pathways may be affected based on concentrations, and a considerable effort is necessary before complete validation of these correlations are obtained, including an appreciation for whatever limits may exist during the correlations. When differences arise between findings of *in vitro* and *in vivo*, the results of *in vivo* shall always take precedence over *in vitro* studies. In many cases, *in vitro* studies which are inexpensive and readily carried out, serve as an adequate screening mechanism that can rule out the importance of a metabolic pathway and make *in vivo* testing reasonably less or even unnecessary .This help the pre-clinical scientist in identifying the behaviour of the compound and the pathway of metabolism aiding it to move on to the next step in the drug discovery

The integration of *in vitro* metabolic studies into a drug-discovery workflow fulfills few important objectives

- ✓ The first is a concerted effort between drug metabolism and medicinal chemistry groups to identify metabolism-related liabilities of compounds in a timely manner, where the effective use of the information helps in managing the evolution of a lead series.
- ✓ while the second is to continually evolve with the practices in accordance with changing regulatory requirements, technological improvements and knowledge, that lies between drug metabolism and toxicology.

This dynamic paradigm is expected to assist in the advancement of drug candidates with a lowered metabolism-related liabilities and commensurately improved chances for success in the clinic. Metabolite profiling the drug phenotype study using specific CYP enzymes would selectively demonstrate the metabolic pathway and the DDI of the drug without the usage of animal models. The results obtained from these studies are redefining the drug Candidates chemistry and structure as per the safety requirements. Thus the early assessment of these drugs metabolism studies in the drug discovery help in optimizing and increasing the success rate of the molecules. Ultimately, it is hoped that these changes will expedite the delivery of effective and safe new medicines to the patients who need them.

**References**


1. Tiller PR, Yu S, Bateman KP, Castro-Perez J, McIntosh IS, Kuo Y, Baillie TA. The role of drug metabolism in drug discovery and development, Rapid Commun. Mass Spectrom. 2008; 22:3510-3516.
2. Baranczewski P, Stańczak A, Kautiainen A, Sandin P, Edlund PO. Introduction to early *in vitro* identification of metabolites of new chemical entities in drug discovery and development. Pharmacol Rep. 2006; 58(3):341-52.
3. Zhang H, Yang Y. New Horizons in Predictive Drug Metabolism and Pharmacokinetics. J. Mass Spectrom. 2008; 43:1181-1190.
4. Ma S, Chowdhury S. Drug Metabolism in Drug Design and Development: Basic Concepts and Practice, John Wiley & Sons, Inc., Hoboken, NJ. 2007; pp. 319-367.
5. Mingshe Zhu, Haiying Zhang, Griffith W. Drug Metabolite Profiling and Identification by High-resolution Mass Spectrometry. J. Biol. Chem. 2011; 286:25419-25425.
6. Dispos Wen B, Fitch WL. Expert Opin. Drug Metabolism in Preclinical Drug Development: Drug Metab. Toxicol 2009; 34:267-280; 5:39-55.
7. Chen G, Daaro I, Pramanik BN, Piwinski JJ. Metabolite Identificaion by Mass Spec. J. Mass Spectrom. 2009; 44:203-213.
8. Dierks EA, Stams KR, Lim HK, Cornelius G, Zhang H, Ball SE. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450s using *in vitro* cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry' Drug. Metab. Dispos. 2001; 29:23-29.
9. Guidance for Industry Safety Testing of Drug Metabolites; U.S. Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER), Pharmacology and Toxicology, 2008 and 2016; Rev.1.
10. Zhu M, Ma L, Zhang D, Ray K, Zhao W, Humphreys WG, *et al*. Involvement of Cytochrome P450 in the *in vitro* metabolism' Drug Metab. Dispos. 2006; 34:1722-1733.
11. Sai Y, Dai R, Yang TJ, Krausz KW, Gonzalez FJ, Gelboin HV, *et al*. Assessment of eight chemical inhibitors using cDNA-expressed cytochromes P450. Xenobiotica. 2000; 30:327-343.
12. Lourette N, Smallwood H, Wu S, Robinson EW, Squier TC, Smith RD, *et al*. Drug Metabolite Profiling and Identification by High-resolution Mass Spectrometry. J. Am. Soc. Mass Spectrom. 2010; 21:930-39.
13. Igho J Onakpoya, Carl J Heneghan, Jeffrey K Aronson. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC Medicine Feb 4, PMC US National Library of Medicine National Institute of Health, 2016.
14. Rhian McNaughton, Gwenaël Huet, Saad Shakir. An investigation into drug products withdrawn from the EU market between 2002 and 2011 for safety reasons and the evidence used to support the decision-making' Drug Safety Research Unit, Southampton, UK, 2013.

15. Maurer HH. Perspectives of liquid chromatography coupled to low- and high-resolution mass spectrometry 'Therapeutic D. Monitoring. 2010; 32:324-327.
16. Wolters Kluwer Lippincott Williams & Wilkins, FOYE'S Principles of Medicinal Chemistry, Drug Metabolism, 6th ed., 2008; 253-325.
17. Perry RH, Cooks RG, Noll RJ. Spectral Accuracy of Molecular ions' Mass Spectrom. Rev. 2008; 27:661-699.
18. Bristow AW. Accurate Mass Measurement for the Elemental formula, Mass Spectrom. Rev. 2006; 25:99-111.
19. Zhu P, Tong W, Alton K, Chowdhury S. Combined Data Mining Strategy for the Systematic Identification.... Anal. Chem. 2009; 81:5910-5917.
20. Ruan Q, Zhu M. Characterisation of Biotransformation enzymes. Chem. Res. Toxicol. 2010; 23:909-917.
21. Zhu M, Ma L, Zhang D, Ray K, Zhao W, Humphreys WG, *et al*. Involvement of Cytochrome P450 in the *in vitro* metabolism. Drug Metab. Dispos. 2006; 34:1722-1733.
22. Sanders GM, Zhang H. A unified model for predicting human hepatic, metabolic clearance from *in vitro* intrinsic clearance data in hepatocytes and microsomes, (34):1722-1733. J. Drug Metab. Dispos. 2006; 33(9):1304-11.
23. Li AC, Ding J, Jiang X, Denissen J. A Decade in the MIST: Learnings' Rapid Commun. Mass Spectrom. 2009; 23:3003-3012.
24. Castro-Perez J, Plumb R, Liang L, Yang E. Metabolite Safety in Drug Testing' Rapid Commun. Mass Spectrom. 2005; 19:798-804.
25. Timothy W Harper, Patrick J Brassil. Reaction Phenotyping: Current Industry Efforts to Identify Enzymes Responsible For Metabolizing Drug Candidates; Department of Metabolism and Pharmacokinetics; Bristol-Myers Squibb. AAPS. 2008; 10:200-207.
26. Chris D Bostick, Katherine M Hickey, Lance A Wollenberg, Darcy R Flora, Timothy S Tracy, Peter M Gannett. Immobilized Cytochrome P450 for Monitoring of P450-P450. Interactions and Metabolism. 2016; 44(5):741-749.
27. Baillie TA. Metabolism and Toxicity of Drugs. Two Decades of Progress in Industrial Drug Metabolism' Chem. Res. Toxicol. 2008; 21:129-137.
28. Ma S, Chowdhury SK, Alton KB. Predictive Toxicology – From vision to reality. Curr. Drug Metab. 2006; 7:503-523.