IJBPAS, April, 2022, 11(4): 1684-1694

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

# RECENT TRENDS IN PHARMACEUTICAL ANALYTICAL CHEMISTRY AS A MAJOR PART OF PHARMACEUTICAL QUALITY CONTROL

**GORAD R* AND CHAUDHARI P**

Bharati Vidyapeeth's College of Pharmacy, Kolhapur, M.S., India

***Corresponding Author: Rajashri Gorad: E Mail: rajashrigorad6@gmail.com**

**Received 24th May 2021; Revised 16th June 2021; Accepted 15th July 2021; Available online 1st April 2022**

https://doi.org/10.31032/IJBPAS/2022/11.4.5996

## ABSTRACT

Analysis of pharmaceutical and natural compounds and newer drugs is commonly used in all the stages of drug discovery and development process. Most utilized chromatographic methods are HPLC and HPTLC. High-performance thin layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin layer chromatography, while the HPLC is the most useful tool in quality control of Pharmaceuticals. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on. Chromatographic methods development and validation play important roles in the discovery, development and Manufacture of pharmaceuticals, drugs and excipients. Method development is the process of proving that a chromatographic method is for used to measure the concentration of drugs, excipients and in biological samples which allow simplified procedures to be employed to verify that an analysis procedure, accurately and consistently will deliver a reliable measurement of an pharmaceutical preparation and bioanalysis. The chromatographic method validation is essential for analytical and bioanalytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness.

**Keywords: HPLC, Drug Metabolism, Drug Discovery, Quality Control**

## INTRODUCTION

Drug metabolism and pharmacokinetic (DMPK) analysis was playing an important role in drug design and development. Measurement of the DMPK profile will lead to early assessment of the drug candidates at the drug development stages, which can be utilized for better structural changes and scaffold design. New technologies and methodologies are imperative to make the drug metabolism and pharmacokinetic screening process more effective. Absorption, distribution, metabolism, and excretion (ADME) studies are important process for drug discovery and development. ADME studies are conducted with *in vitro* , *in vivo* or *in silico* models. *In vitro* models generate many ADME parameters, including apparent permeability, metabolic stability, reaction phenotyping, protein binding, blood to plasma partitioning, drug – drug interaction potentials (e.g inhibition and induction of Cytochrome P450 and transporters), cell proliferation and cytotoxicity. *In vivo* models of animal and healthy human subjects provide information such as drug oral bioavailability, exposure, distribution, clearance, and duration of exposure for a drug and its metabolites. *In silico* models predict drug behaviors based on physicochemical properties of drug candidates in combination with crystal structure of protein **[1]**.

New technologies and methodologies are imperative to make the DMPK screening process more effective. The definitions of metabolic profiling include the measurement of the dynamic multi parametric metabolic response of living system to pathophysiological stimuli or genetic modification. Metabolic profiling is process of measurement of metabolites and their intermediates in the biological systems. These biological systems generally include the urine, serum, or biological tissue extract, which gave numerous opportunities of effect of external or internal stimuli. Toxicity assessment is another application of metabolic profiling which can give ideal insight mechanistic detail about structural about changes leading to the toxicities **[2]**.

Human liver is main metabolizing organ in human systems comprised of number of oxidative enzymes like Cytochrome P450 enzymes. Human CYP are major metabolizing enzymes sub classified in to number of sub type like CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, and CYP4A11. The process of metabolic is a

chemical conversion of drug to transforms lipophilic drug into more polar, hydrophilic, readily excretable product **[3]**.

A xenobiotic that is metabolized by particular enzymes may become a competitor for endogenous substances, which is metabolized by same enzyme. A substance that is absorbed orally from the gastrointestinal tract (GIT) is transported via the portal circulation to the liver, where it may be subjected to first pass hepatic metabolism, followed by elimination in the bile or urine via the kidneys. There is also the possibility of extra-hepatic metabolism. A typical chemical metabolism pathway involves the oxidation of the parent substance (phase I oxidation), the final product usually contains a chemical reactive functional group OH, $NH_2$, SH, COOH, followed by conjugation of the oxidized moiety with highly polar molecules, such as glucose, sulphate, methionine, cysteine, glutathione or glucuronic acid (phase II conjugation). The key enzymes for phase I oxidation are the isoforms of the CYP family of enzymes. The phase I oxidative enzymes are almost exclusively localized in the endoplasmic reticulum. While the liver is the main site of xenobiotic metabolism, phase I and phase II metabolism also occurs in most tissues including the gut microflora and usually results in loss of pharmacological activity. Polymorphisms and inter-individual variability in the expression of these enzymes are a key reason why different individuals react differently to chemicals, and also a major reason for the wide range of experimental variability with tests based on liver tissue **[4]**.

Metabolism usually occurs in the liver, but the enzymes (especially CYP450 3A4) also are important in gut metabolism. Human liver microsomes provide the most convenient way to study CYP450 metabolism. Microsomes are a subcellular fraction of tissue obtained by differential high-speed centrifugation. All of the CYP450 enzymes are collected in the microsomal fraction. The CYP450 enzymes retain their activity for many years in microsomes or whole liver stored at low temperature **[5]**.

**SIGNIFICANCE OF METABOLIC PROFILING IN DRUG DISCOVERY**

- When the drug reaches their specific target site it will metabolism and produce desire specific therapeutic effect.
- In Administration more than one drug affected phase I interaction this known as drug-drug interaction, in administration various drug can affected

by inhibition or induction of cytochrome.

- The drug metabolism is a major criterion in the high-throughput screening of prospective drugs. Drug metabolism has an important role in the determination of the pharmacokinetic (PK) parameters like half life, oral bioavability and clearance of the entity within the cell.
- When the drug metabolism affected in proper action it will be longer period of time it will be easily eliminated and inhibited in longer period of time and producing toxic effect.
- The drug-drug interaction study has become a fraction of the metabolic stability study of a prospective drug.
- The determination of the metabolite structure with the help of LC/MS-MS and the metabolic phenotypin e.g. CYP 450 it is prominent example of the drug metabolism it will the retain the activity of microsomes **[6-8]**.

## FACTORS AFFECTING XENOBIOTIC METABOLISM [9]

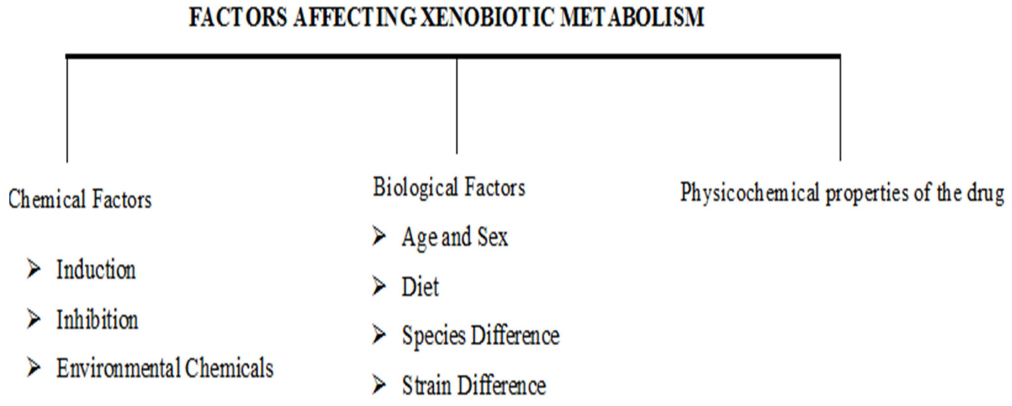


**Figure 1: Factors Affecting Xenobiotic Metabolism**

## CHEMICAL FACTORS [10]

### Induction

The Phenomenon of increased drug metabolizing ability of enzymes by several drugs and chemicals is called as enzyme induction and the agents which bring about such an effect are called enzyme inducer.

### Mechanism of enzyme Induction

- Increased stability of CYP-450
- Increased stability of enzymes
- Increase microsomal protein content
- Decreased degradation of CYP-450

### Consequences of Induction

- Decrease in pharmacological activity of drugs
- Decrease in drug plasma concentration
- Increased rate of metabolism

- Reduced bioavailability

**Inhibition**

A decrease in the drug metabolizing ability of an enzyme is called as enzyme inhibition. The process of inhibition may be direct or indirect

**Direct Inhibition :** It may be result from interaction at the enzymatic site, the net outcome being a change in enzyme activity.

**Mechanism of the direct inhibition**

1. Competitive inhibition
2. Non- Competitive inhibition
3. Product inhibition

**Indirect Inhibition: Irreversible** type of inhibitors made enzyme inactive by making complex with heme iron of CYP450, or destruction of heme group.

**Mechanism of the indirect inhibition**

1. Repression
2. Altered physiology

**Consequences of Inhibition**

- Reduction in metabolite concentration.
- Increased risk of drug-induced toxicity.
- Increase in the plasma concentration of parent drug.

**Environmental Chemicals**

The environmental agents influence the drug metabolizing ability of enzymes.

**2. Biological factor**

**Age and sex**

The drug metabolic rate in the different age groups differs mainly due to variation in the enzyme content, enzyme activity and hemodynamic. The variations between male and female are observed. Sex related differences in the rate of metabolism may be due to sex hormones.

**Diet**

The activity and enzyme content is altered by a number of dietary components. High protein diet increase and low protein decreases the drug metabolizing ability. .

**Species Difference**

Species Difference has been observed in both Phase-I and phase-II reaction. In the phase-I reaction, both quantitative and qualitative variations in the enzyme and their activity has been observed.

**Strain difference**

The difference in the drug metabolizing ability between different species is attributed to genetics, the differences are observed between same species. It may be studied

- Pharmacogenetics
- Ethnic variation

**Physicochemical properties of the drug**

Molecular size and shape, pka, lipophilicity, acidity/basicity, steric and electronic characteristics of a drug influence in interaction with the active site of enzyme. The therapeutic efficacy, toxicity, and

biological half-life of a drug depend on the metabolism of the drug and a number of factors affect the metabolism of the drug.

**DRUG METABOLISM**

Drug metabolism is the biochemical modification of pharmaceutical substances or xenobiotics respectively by living organisms, usually through specialize enzymatic systems **[11-13]**.

Drug metabolism is divided into three phases.

- **Phase I Reactions**

  Convert parent compound into a more polar (hydrophilic) metabolite by adding or unmasking functional groups. e.g. –SH, -OH,-COOH,-$NH_2$ etc.

- **Phase II Reaction**

  This reaction is also called as conjugation reaction. Conjugation with endogenous substrate to further increase aqueous solubility.

- **Phase III Reaction**

This reaction is in further modification and excretion.

Liver is the main site of xenobiotic metabolism i.e. phase I and phase II metabolism as liver contains number of metabolizing enzymes.

**Table 1 : Phase I and Phase II Reaction involved in drug metabolism**

| Phase | Reaction | Enzyme |
|---|---|---|
| | Oxidation of aliphatic and aromatic groups | Cytochrome P450 |
| I | Reduction of azo and nitro groups | Flavin enzymes |
| I | Hydrolysis | Esterases, Amidases |
| II | Glucuronide synthesis | Glucuronyl transferase |
| II | Methylation | Catechol-O-methyl tranferase |
| II | Acetylation | N-acetyl trasferase |

**CHROMATOGRAPHY**

A Russian botanist Mikhail Tswett 1872-1919 is accredited with the first use of chromatography in 1906 when he separated plant pigments such as chlorophylls and xanthophylls. Chromatography is an analytical that is widely used for the separation of components in a mixture due to the differing time taken for each component to travel through a stationary phase when carried through it by a mobile phase **[14]**.

Chromatography is defined as the method of separating a mixture of component into individual components through equilibrium distribution between two phase (stationary phase) (mobile phase) **[15]**.

**Classification of Chromatographic methods**

Chromatographic techniques can be classified according to whether the known as separation takes place on a planar surface or in a column. They can be further sub-divided into gas and liquid chromatography, and by the physical form, solid or liquid **[16]**.

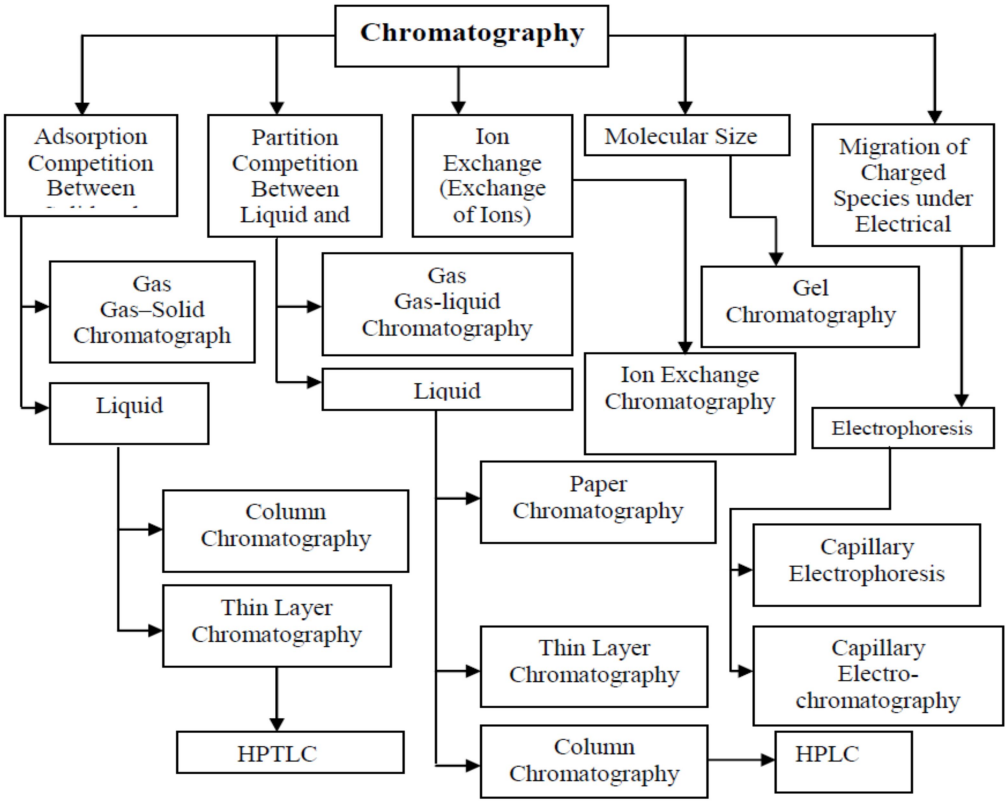


**Figure 2: Classification of chromatographic methods**

## HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is a separation technique where solutes migrate through a column containing a micro particulate stationary phase at rates dependent on their distribution ratios. These are functions of the relative affinities of the solutes for the mobile and stationary phases, the elution order depending on the chemical nature of the solutes and the overall polarity of the two phases. It is a versatile analytical technology widely used for the analysis of pharmaceuticals, biomolecules, polymers, and many organic and ionic compounds **[17, 18]**.

### Types of HPLC

There are several ways to classify liquid column chromatography. According to nature of the chromatography, stationary phase and the separation process, three modes can be specified.

- Adsorption chromatography.
- Ion-exchange chromatography.
- Size exclusion chromatography.
- Ion-pair chromatography.

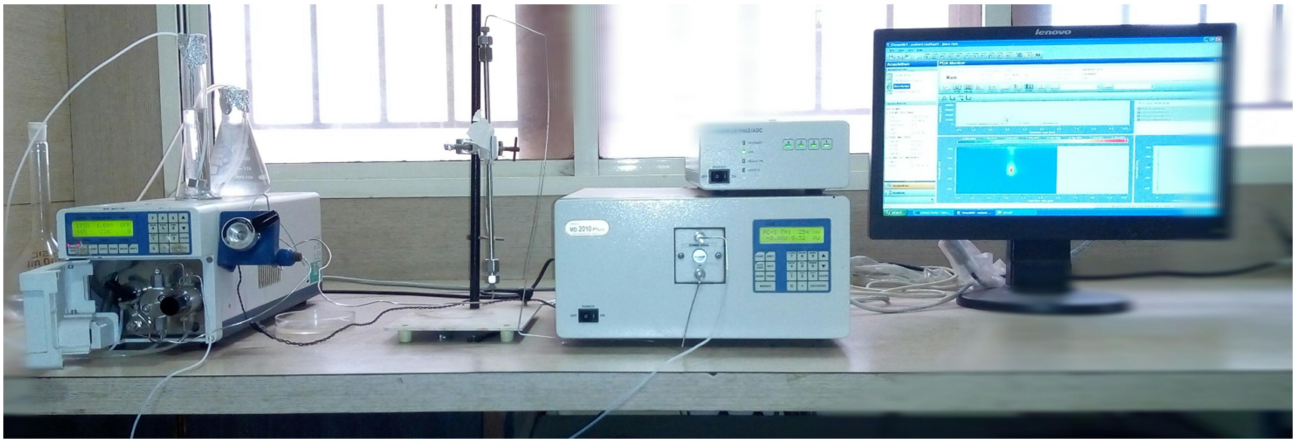

**Figure 3: Instrument of HPLC**

## VALIDATION OF ANALYTICAL METHOD

Validation is defined as the "establishing of documented evidence which provides a high degree of assurance that a planned process will consistently perform according to the intended specified outcomes" Validation studies are performed for analytical tests, equipment, facility systems such as air, water, steam and for processes such as the manufacturing processes, cleaning, sterilization, sterile filling, lyophilization etc **[19, 20]**.

### Objective of Validation

- Validation is an evaluation of whether the precision and accuracy obtained by following the procedure are appropriate for the problem.
- Validation also ensures that the written procedure has sufficient feature so that different analysts or laboratories following the same procedure obtain comparable results.

### Validation parameters [21]

- **Linearity:** Checked the response for the drug whether it is linear in the concentration range of 10-50 µg/ml.
- **Precision:** Intra- and inter-day precision studies were carried out by injecting three replicates standard solution of Vemurafenib (10, 30 and 50 µg/ml) .
- **Accuracy:** Recovery studies by spiking different concentrations of pure drug (80%, 100% and 120%).
- **LOD:** The limit of detection is defined as the lowest concentration of an analyte in a sample that can be detected, not quantitated. LOD = 3.3 σ/S.
- **LOQ:** Based on the Standard Deviation of the Blank: Measurement

of the magnitude of analytical background response was performed by analyzing the three replicates of blank samples and calculating the standard deviation of these responses. LOQ = 10 σ/S.

- **Ruggedness:** Ruggedness, according to the USP, is the degree of reproducibility of the results obtained under a variety of conditions, expressed as % RSD.

## DOCKING STUDIES

Recently, drug discovery has significantly increased due to the availability of 3D X-ray or NMR structures of biomolecule, docking tools, and the development of computer aided drug design (CADD) methodologies. Similarly, number of lead drug like molecules is also relatively less. Thus an improved approach of rational drug design is necessary to overcome problems associated with currently available drugs that are developed based on the sole approach of structure guided drug design **[22]**.

Drug or ligand design, is the inventive process of finding new medications on the knowledge of a biological target. The drug is an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient **[23]**.

## CONCLUSION

A Brief review has been studied based on existing literature for the availability of experimental tools which are essential for analytical and bioanalytical method development and tested extensively for specificity, linearity, accuracy, precision, range, detection limit, quantization limit, and robustness. This review, will surely be useful for the researchers in quality control of Pharmaceuticals.

## REFERENCES

**[1]** Clarke CJ, Haselden JN. Metabolic profiling as a tool for understanding mechanisms of toxicity. Toxicologic pathology. 2008 Jan; 36(1): 140-7.

**[2]** Sun T, Chen S, Huang H, Li T, Yang W, Liu L. Metabolic profile study of 7, 8-dihydroxyflavone in monkey plasma using high performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography B. 2017 Sep 1; 1061: 97-102.

**[3]** Huang J, Bathena SP, Alnouti Y. Metabolite profiling of praziquantel and its analogs during the analysis of in vitro metabolic stability using information-dependent acquisition on a hybrid triple-quadrupole linear ion trap mass spectrometer. Drug metabolism and pharmacokinetics. 2010: 1009210037.

**[4]** Zhang W, Qiu KX, Yu F, Xie XG, Zhang SQ, Chen YJ, Xie HD. Virtual screening of

B-Raf kinase inhibitors: A combination of pharmacophore modelling, molecular docking, 3D-QSAR model and binding free energy calculation studies. Computational biology and chemistry. 2017 Oct 1; 70: 186-90.

**[5]** Spalding DJ, Harker AJ, Bayliss MK. Combining high-throughput pharmacokinetic screens at the hits-to-leads stage of drug discovery. Drug discovery today. 2000 Dec 1; 5(12): 70-6.

**[6]** Di L, Kerns EH. Profiling drug-like properties in discovery research. Current opinion in chemical biology. 2003 Jun 1; 7(3): 402-8.

**[7]** Kerns EH, Di L. Pharmaceutical profiling in drug discovery. Drug discovery today. 2003 Apr 1; 8(7): 316-23.

**[8]** Alavijeh MS, Palmer AM. The pivotal role of drug metabolism and pharmacokinetics in the discovery and development of new medicines. Curr. Opin. Investig. Drugs. 2004; 5: 755-63.

**[9]** Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. New England Journal of Medicine. 2011 Jun 30; 364(26): 2507-16.

**[10]** Dummer R, Rinderknecht J, Goldinger SM. Ultraviolet A and photosensitivity during vemurafenib therapy. The New England journal of medicine. 2012 Feb 1; 366(5): 480-1.

**[11]** Wu CP, Sim HM, Huang YH, Liu YC, Hsiao SH, Cheng HW, Li YQ, Ambudkar SV, Hsu SC. Overexpression of ATP-binding cassette transporter ABCG2 as a potential mechanism of acquired resistance to vemurafenib in BRAF (V600E) mutant cancer cells. Biochemical pharmacology. 2013 Feb 1; 85(3): 325-34.

**[12]** Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999 Jan 1; 29(11): 1181-9.

**[13]** Zhou S, Chin R, Kestell P, Tingle MD, Paxton JW. Effects of anticancer drugs on the metabolism of the anticancer drug 5, 6-dimethylxanthenone-4-acetic (DMXAA) by human liver microsomes. British journal of clinical pharmacology. 2001 Aug; 52(2): 129-36.

**[14]** Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB. Inhibition of mutated, activated BRAF in metastatic melanoma. New England Journal of Medicine. 2010 Aug 26; 363(9): 809-19.

**[15]** Zhen Y, Thomas-Schoemann A, Sakji L, Boudou-Rouquette P, Dupin N, Mortier L, Vidal M, Goldwasser F, Blanchet B. An HPLC-UV method for the simultaneous

quantification of vemurafenib and erlotinib in plasma from cancer patients. Journal of Chromatography B. 2013 Jun 1; 928: 93-7.

**[16]** Sparidans RW, Durmus S, Schinkel AH, Schellens JH, Beijnen JH. Liquid chromatography–tandem mass spectrometric assay for the mutated BRAF inhibitor vemurafenib in human and mouse plasma. Journal of Chromatography B. 2012 Mar 15; 889: 144-7.

**[17]** Nijenhuis CM, Rosing H, Schellens JH, Beijnen JH. Development and validation of a high-performance liquid chromatography–tandem mass spectrometry assay quantifying vemurafenib in human plasma. Journal of pharmaceutical and biomedical analysis. 2014 Jan 25; 88: 630-5.

**[18]** Gibson. G, Skett, P. Pathways of drug metabolism. In Introduction to Drug Metabolism. *Nelson Thornes Publishers: Cheltenham*, UK, 2001. 1–36.

**[19]** Svenson J, Vergote V, Karstad R, Burvenich C, Svendsen JS, De Spiegeleer B. Metabolic fate of lactoferricin-based antimicrobial peptides: effect of truncation and incorporation of amino acid analogs on the in vitro metabolic stability. Journal of Pharmacology and Experimental Therapeutics. 2010 Mar 1; 332(3): 1032-9.

**[20]** Roberts SA. High-throughput screening approaches for investigating drug metabolism and pharmacokinetics. Xenobiotica. 2001 Jan 1; 31(8-9): 557-89.

**[21]** Lin JH, Lu AY. Inhibition and induction of cytochrome P450 and the clinical implications. Clinical pharmacokinetics. 1998 Nov; 35(5): 361-90.

**[22]** White RE. High-throughput screening in drug metabolism and pharmacokinetic support of drug discovery. Annual review of pharmacology and toxicology. 2000 Apr; 40(1): 133-57.

**[23]** Adams Jr J, Baillie TA, Trevor AJ, Castagnoli Jr N. Studies on the biotransformation of ketamine 1—identification of metabolites produced in vitro from rat liver microsomal preparations. Biomedical mass spectrometry. 1981 Nov; 8(11): 527-38.