# Metabolic Studies of Drug Candidates for Neurological Disorders and Asthma Based on $GABA_A$ Receptor Subtype Selective Ligands using Mass Spectrometry

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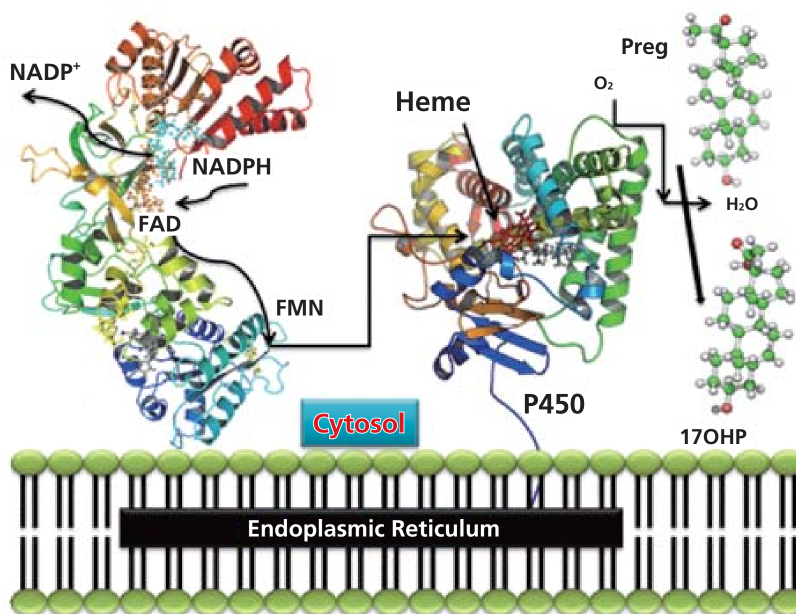
## Abstract

Development of pre-clinical experimental models to understand the *in vivo* metabolic performance of a drug is important in the field of drug discovery. GABA-ergic drugs are historically used for the treatment of neurological disorders such as neuropathic pain, schizophrenia and anxiety but recently have shown potential to treat asthma. In the present study, an *in vitro* microsomal assay was designed to evaluate the metabolic stability of $GABA_A$ receptor subtype selective ligands using microsomes and S9 fractions of human and mouse liver extracts. A LC-MS/MS method was developed to quantify the amount of drug degrading over a period of time using verapamil as internal standard. Herein, we will report the development, analysis and standardization of a liver microsome stability assay using the Shimadzu LCMS-8040 triple quadrupole instrument at the MIDD.

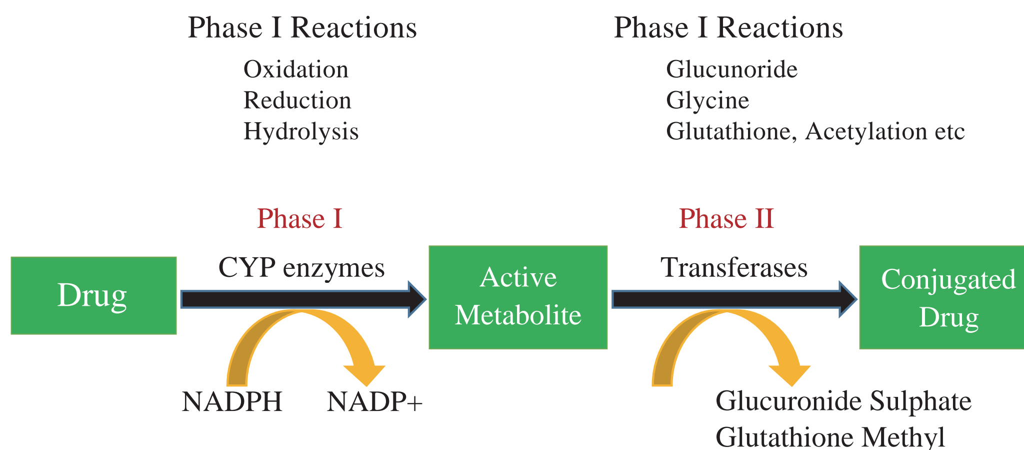
## Introduction

Drug metabolism is process of converting hydrophobic xenobiotic to highly water soluble species by biochemical modification, facilitating the elimination of drugs from the body. Metabolic stability refers to susceptibility of drugs to bio-transformational enzymes such as cytochrome P450, which are abundant in the liver. Microsomes and S9 fractions are subcellular fractions of liver tissue. Microsomes are vesicles derived from the endoplasmic reticulum containing CYP 450 enzymes responsible for phase I biotransformation reactions. The S9 fraction is a mixture of microsomes and cytosol containing both phase I and Phase II metabolic enzymes.



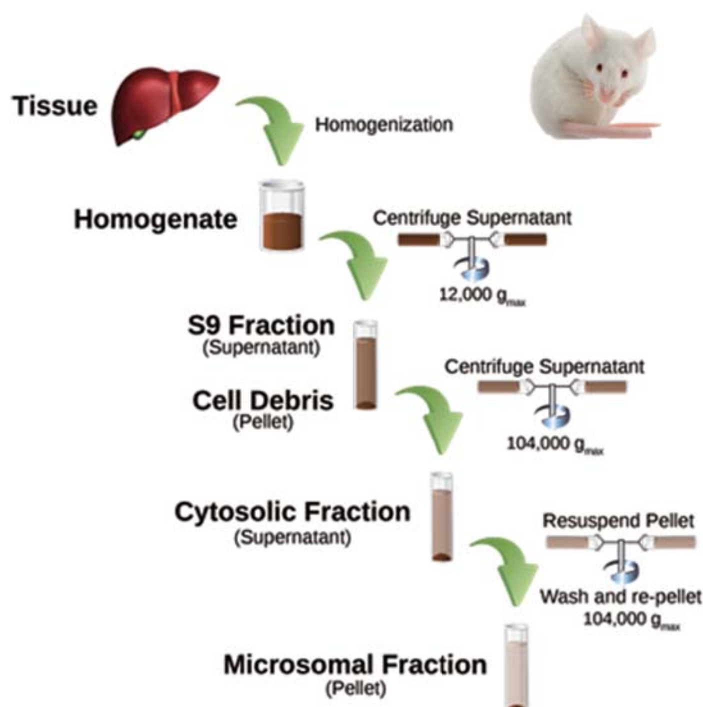

CYP 450 system

### Phases of metabolism




### Studies in different animal species

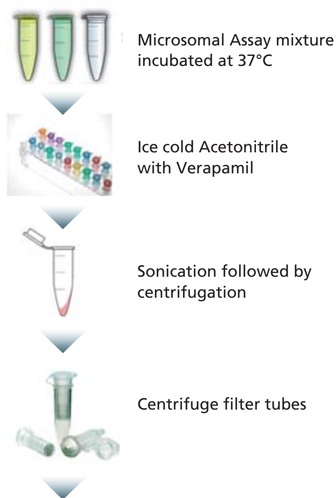
The isoform composition and catalytic activity of cytochrome P450 enzymes varies for different animal species. Therefore, *in vitro* metabolic studies are carried out with micosomes derived from different animals and humans to identify interspecies variation. The P450 isoform CYP3A4 is known to be mainly involved in metabolism of xenobiotic. Microsomes and S9 are obtained by differential high speed centrifugation from liver homogenate.



## Protocol

### Incubation conditions

- Test Compound concentration: 10 μM
- Microsomal Protein concentration: 0.5 mg/mL
- Incubation time: 60 min
- Co-factors added: NADPH
- Internal standard: Verapamil
- Positive control: HZ-166
- Negative control: without microsomes
- Time points: 0,10,20,30,40,50 and 60 min
- Instrument: Digital Dry bath
- Temperature: 37°C




Analysis by LCMS 8040

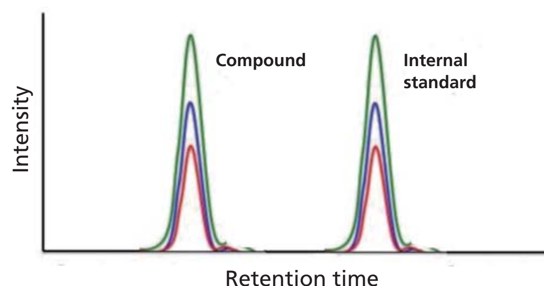
## Analytical method

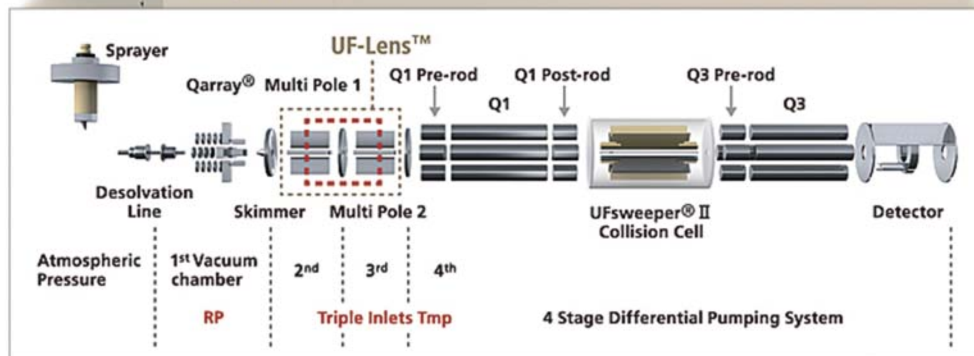
A LC-MS/MS method is developed for each compound. In addition, a calibration curve is established to quantify the amount of compound during metabolism. Two approaches are used.

- Disappearance of the parent ion mass over a period of time
- Accumulation of the metabolites over a period of time

Different SCAN and SIM modes of the quadrupoles useful in the analysis

- Q3 Scan
- Selective Ion Monitoring
- Product ion scan
- MRM Transition

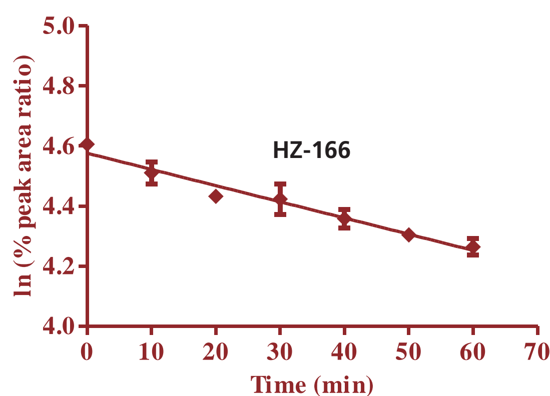


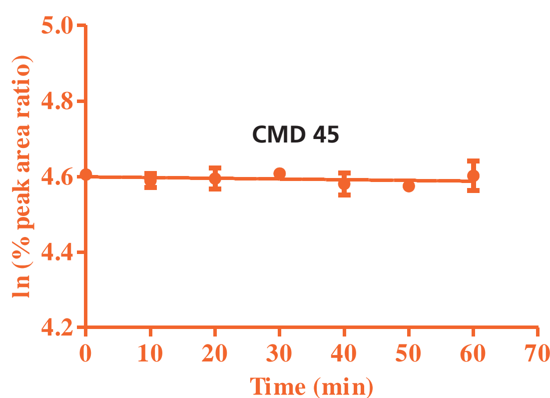


## Data analysis HZ-166

Peak area and % remaining values

| Time (min) | Test compound Peak area | Verapamil Peak area | % remaining |
|---|---|---|---|
| 0 | 815599 | 586830 | 99.98 |
| 10 | 702717 | 543057 | 87.53 |
| 20 | 693332 | 550156 | 84.60 |
| 30 | 655521 | 515666 | 80.23 |
| 40 | 608029 | 558567 | 76.24 |
| 50 | 604208 | 566352 | 74.73 |
| 60 | 561988 | 561427 | 69.06 |



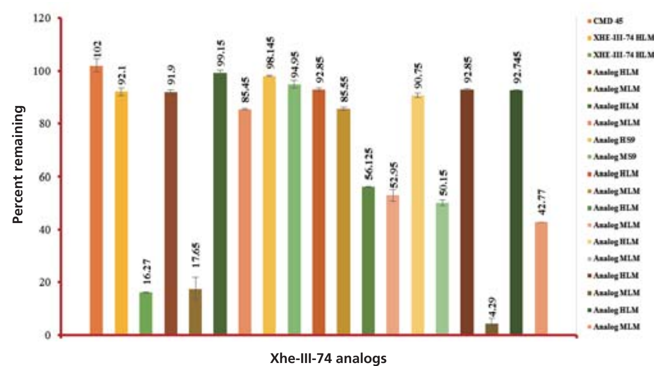

## Metabolic parameters

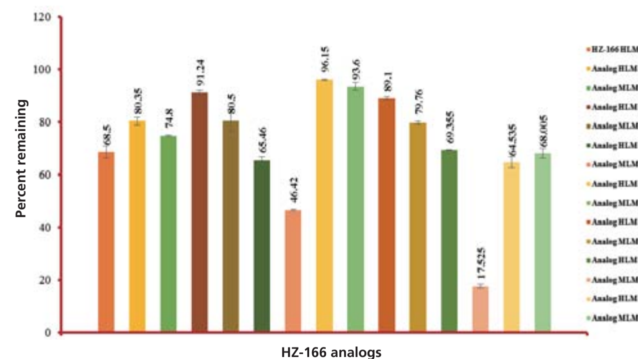
$$t_{1/2} = \frac{0.693}{\text{slope}}$$

$$\text{Intrinsic clearance} = \frac{(V*0.693)}{t_{1/2}}$$

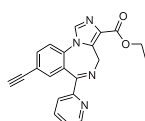
$$V = \frac{\text{incubation volume}}{[\text{protein}]}$$

$$\text{Metabolic rate} = \frac{\text{slope}*[\text{analyte}]}{[\text{protein}]}$$

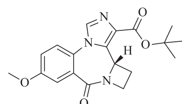


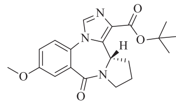

## Compounds analyzed



**HZ-166**
Chemical Formula: $C_{21}H_{16}N_4O_2$
Molecular Weight: 356.38



**CDM-45**
Chemical Formula: $C_{19}H_{21}N_3O_4$
Molecular Weight: 355.39



**Xhe-III-74**
Chemical Formula: $C_{20}H_{23}N_3O_4$
Molecular Weight: 369.41

## Conclusion and future

During our investigation, we observed that mouse microsomes degrade compounds more quickly than human microsomes. We are currently in process to characterize these metabolites in order to guide the synthesis of new analogs for the research group of Prof. Cook. We will expand this method to pharmacokinetic studies in the near future.

## Acknowledgements

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