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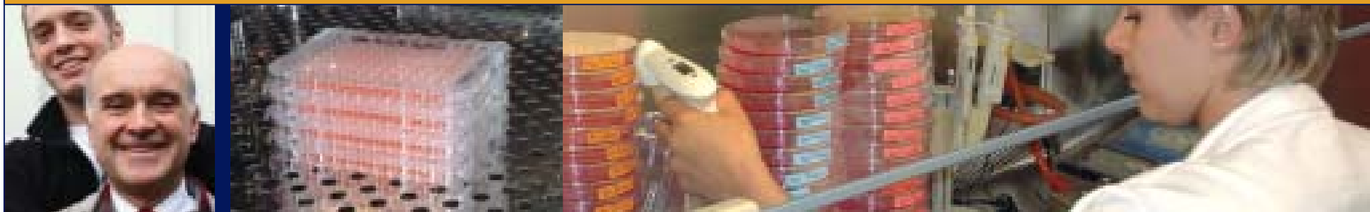
in vitro / in vivo

"Individual expertise in finding solutions at the preclinical stage and a high level of reliability are strengths we particularly appreciate in GenPharmTox for a partnership-based collaboration."

Dr. Michael Gassen, 4SC AG

2 ADME *in vitro / in vivo*



In vitro Services

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In vivo Services

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In vitro Services

2.1. Absorption

2.1.1. Caco-2 Permeability Assay

Caco-2 cells are a well-established *in vitro* model to predict intestinal absorption of drugs and chemicals in humans.
Plated Caco-2 cells on a membrane support allow the study of drug transport from the apical side to the basolateral side (A to B transport) as well as from the basolateral side to the apical side (B to A transport). The apical and basolateral sides of Caco-2 cells represent the luminal and blood sides, respectively, of the gastrointestinal tract *in vivo*. In addition, the transport kinetics can be investigated.
The apparent permeability (P_{app}) of the test item is calculated and compared with that of reference compounds of known permeability.

Quantity of test item required: 15 mg
Turnaround time of draft report: 20 working days

Transport
- triplicates
- Caco-2 cells (monolayer in Transwell® plates)
- one concentration of test item (50 µM)
- one time point (120 min)

Kinetics
- triplicates
- Caco-2 cells (monolayer in Transwell® plates)
- one to four concentrations of test item (12.5, 25, 50, 100 µM)
- one to six time points (0, 15, 30, 60, 90, 120 min)
- positive controls

Controls
- propranolol (high permeability, transcellular transport)
- hydrocortisone (moderate permeability, transcellular transport)
- ranitidine (low permeability, paracellular transport)
- vinblastine inhibited by verapamil (P-glycoprotein mediated transport)
- measurement of the transepithelial electrical resistance (TEER) to check for membrane integrity

Analytics
- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: detection of parent compound

2.2. Metabolic Stability

The metabolic stability of the test item is determined using liver microsomes or primary hepatocytes of different species. The metabolic stability is also determined during "Metabolite Profiling and Species Comparison".

2.2.1. Metabolic Stability as Drug Discovery Screen

The "percentage metabolism" of the test item is determined.

Quantity of test item required: 1 mg (pre-weighted)
Turnaround time of draft report: 5 working days

Study Design

- duplicates
- 0.5 mg/ml of human and rat liver microsomes or 0.5 x 10^6 hepatocytes/ml
  Please inquire for further species.
- one concentration of test item (10 µM)
- one time point (60 min)
- positive control: formation of 7-hydroxycoumarin
- negative control: zero time point or no NADPH

Analytics

- LC-MS/MS detection (loss of parent compound)

2.2.2. Metabolic Stability as Drug Development Assay

The "percentage metabolism" and the Cl_{int} and $t_{1/2}$ of the test item are determined.

Quantity of test item required: 5 mg (pre-weighted)
Turnaround time of draft report: 20 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of human and rat liver microsomes or primary hepatocytes
  Please inquire other species.
- one concentration of test item
- five time points
- positive control: formation of 7-hydroxycoumarin
- negative control: zero time point or no NADPH

Analytics

- optimised LC-MS/MS detection (loss of parent compound, metabolite profiling possible)

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2.3. CYP Profiling

Identification of CYP isoforms involved in the metabolism of the test item *in vitro*.

2.3.1. Human Recombinant Enzymes

The "percentage metabolism" of the test item with respect to different human recombinant CYP isoenzymes is determined

Quantity of test item required: 15 mg
Turnaround time of draft report: 15 working days

Study Design

- 9 CYP isoenzames
  1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4
  Please inquire for further CYP-isoforms.
- triplicates
- one concentration of CYP
- one concentration of test item
- one time point
- positive control: CYP marker reaction
- negative control: non-expressing control

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound.

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2.3.2. Chemical Inhibition

The inhibitory effect of different specific CYP inhibitors on the metabolism of the test item in pooled human liver microsomes is investigated.

Quantity of test item required: 15 mg (pre-weighted)
Turnaround time of draft report: 15 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of pooled human liver microsomes
- one concentration of test item
- one time point
- one chemical inhibitor per CYP isoenzyme
  (one to three concentrations)
- positive control: incubations without inhibitor
- negative control: zero time point and/or no NADPH

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound

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2.3.3. Correlation Analysis

The CYP isoenzymes involved in the *in vitro* metabolism of the test item are identified by correlation analysis using individual human liver microsomes. The inter-individual rate of metabolism of the test item is compared to the variation in the rate of a set of CYP marker reactions. The respective correlation coefficients are determined.

Quantity of test item required: 15 mg
Turnaround time of draft report: 15 working days

Study Design

- range finding (optional)
- triplicates
- 16 individual human liver microsomal samples (one concentration)
- one time point
- positive control: CYP marker reaction
- negative control: zero time point and/or no NADPH

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: loss of parent compound and/or formation of selected metabolites and/or identification of major oxidative metabolites by mass relative to the parent compound

2.4. Enzyme Kinetics

The apparent kinetic constants (K_M, V_{max}, Cl_{int}) are determined using microsomes, primary hepatocytes or recombinant enzymes.

Quantity of test item required: 5 mg
Turnaround time of draft report: 20 working days

Range Finding

- single determinations
- three concentrations of test system (e. g. 0.5, 1, 1.5 mg microsomal protein/ml)
- three concentrations of test item (e. g. 1, 10, 100 µM)
- three time points (e. g. 15, 30, 60 min)
- positive control: enzyme marker reaction (e. g. formation of 7-hydroxycoumarin)
- negative control: zero time point and no NADPH

Study Design

- triplicates
- one concentration of test system
- seven to ten concentrations of test item
- one time point
- positive control: enzyme marker reaction
- negative control: zero time point

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection: detection of loss of parent compound and/or formation of selected metabolites, quantitative

Comment

If no comparable data are available, a range-finding pretest is pre-requisite for the determination of the enzyme kinetics in order to ensure sufficient metabolism of the test item as well as linear response with time and test system concentration. Further, a reference metabolite should be available for calibration and calculation of the V_{max} and $Cl_{int.}$ Alternatively, radiolabelled test item can be used. Indirect calculation by the loss of parent compound is usually limited by insufficient metabolism rates at high test item concentrations.

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2.5. Species Comparison and Metabolite Profiling

The metabolic stability and metabolite profile of the test item is investigated and compared with respect to different species *in vitro*. The data obtained provide valuable information for species selection in toxicological studies *in vivo*.

2.5.1. Available Species, Strains and Test Systems

Species	**Liver Microsomes**	**Primary Hepatocytes**	
		cyropreserved	**freshly isolated**
Human	individual donor or pooled (male, female, mixed)	individual donor or pooled (male, female, mixed)	individual donor (male, female)
Rat	Sprague Dawley Fischer 344 Wistar IGS Sprague Dawley (male, female, mixed)	Sprague Dawley Wistar (male, female, mixed)	Sprague Dawley Fischer 344 IGS Sprague Dawley (male, female, mixed)
Dog	Beagle Dog (male, female, mixed)	Beagle Dog (male, female. mixed)	Beagle Dog (male, female)
Guinea Pig	Hartley Albino (male)	please inquire	please inquire
Rabbit	New Zealand (male)	New Zealand (male, female, mixed)	please inquire
Mouse	CD1 B6C3F1 (male) (male)	CD1	please inquire
Monkey	Rhesus Rhesus Cynomolgus (male, female, mixed)	Cynomolgus Cynomolgus (male, female, mixed)	 (male, female)
Hamster	Golden Syrian (male)	please inquire	please inquire
Pig	please inquire (male)	Gottingen Minipig	individual animal

Please inquire for other species and tissues, gender, or induced animal products.

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2.5.2. Study Outline

Quantity of test item required: 10 mg / species
Turnaround time of draft report: 20 – 30 working days

Study Design

- range finding (optional)
- triplicates
- one concentration of test system
- one concentration of test item
- one to six time points
- positive control:
  formation of 7-hydroxycoumarin and/or
  selected phase I and/or phase II marker reactions
- negative control:
  zero time point and/or no NADPH
- TI blank (no test item)
- TS blank (not test system)
- TITS blank (no test item, no test system)

Analytics

- HPLC-DAD/FLD or LC-MS/MS or Radiodetection:
  loss of parent compound and/or formation of selected metabolites and/or identification of major phase I oxidative metabolites and/or major phase II conjugates by mass relative to the parent compound

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2.6. CYP Inhibition

The inhibiton of CYP isoenzymes is one of the key aspects in drug-drug interaction.

2.6.1. Drug Discovery Screen

The percentage inhibition of different CYP marker reaction activities by the test item is determined.

Quantity of test item required: 1 mg (pre-weighted)
Turnaround time of draft report: 15 working

Study Design

- multiple CYP isoforms (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- 50 µM of test item
- 30 min
- negative controls: CYP marker reactions:
  1A1/2 (7-ethoxyresorufin O-deethylation)
  2A6 (coumarin 7-hydroxylation)
  2B6 (S-mephenytoin N-demethylation)
  2C8 (paclitaxel 6α-hydroxylation)
  2C9 (diclofenac 4'-hydroxylation)
  2C19 (S-mephenytoin 4'-hydroxylation)
  2D6 (bufuralol -hydroxylation)
  2E1 (chloroxazone 6-hydroxylation)
  3A4/5 (testosterone 6ß-hydroxylation)
  4A11 (lauric acid-hydroxylation)
  11B1 (11-deoxycortisol 11ß-hydroxylation)
  11B2 (deoxycorticosterone –hydroxylation)

- negative controls: CYP reference inhibitors:
  1A1/2 (furafylline)
  2A6 (8-methoxypsoralene)
  2B6 (triethylenethiophosphoramide)
  2C8 (ketoconazole)
  2C9 (sulfaphenazole)
  2C19 (omeprazole)
  2D6 (quinidine)
  2E1 (diethyldithiocarbamate)
  3A4/5 (ketoconazole)
  4A11 (10-(imidazolyl)-decanoic acid)
  11B1 (metyrapone)
  11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

2.6.2. Determination of the IC_{50}

The IC_{50} of the test item for a specific CYP marker reaction or drug – drug interaction is determined.

Quantity of test item required: 15 mg
Turnaround time of draft report: 15 working days

Study Design

- one CYP isoform (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- one concentration of marker substrate
- seven to ten concentrations of test item
- one time point
- negative controls: CYP marker reactions:
  1A1/2 (7-ethoxyresorufin O-deethylation)
  2A6 (coumarin 7-hydroxylation)
  2B6 (S-mephenytoin N-demethylation)
  2C8 (paclitaxel 6α-hydroxylation)
  2C9 (diclofenac 4'-hydroxylation)
  2C19 (S-mephenytoin 4'-hydroxylation)
  2D6 (bufuralol-hydroxylation)
  2E1 (chloroxazone 6-hydroxylation)
  3A4/5 (testosterone 6ß-hydroxylation)
  4A11 (lauric acid-hydroxylation)
  11B1 (11-deoxycortisol 11ß-hydroxylation)
  11B2 (deoxycorticosterone –hydroxylation)

- positive controls: CYP reference inhibitors:
  1A1/2 (furafylline)
  2A6 (8-methoxypsoralene)
  2B6 (triethylenethiophosphoramide)
  2C8 (ketoconazole)
  2C9 (sulfaphenazole)
  2C19 (omeprazole)
  2D6 (quinidine)
  2E1 (diethyldithiocarbamate)
  3A4/5 (ketoconazole)
  4A11 (10-(imidazolyl)-decanoic acid)
  11B1 (metyrapone)
  11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

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2.6.3. Determination of the K_i

The K_i of the test item for a specific CYP isoform marker reaction or drug – drug interaction is determined.

Quantity of test item required: 20 mg
Turnaround time of draft report: 20 working

Study Design

- one CYP isoform (microsomes or human recombinant enzymes)
- triplicates
- one concentration of CYP
- three to six concentrations of marker substrate
- three to six concentrations of test item
- one time point
- negative controls: CYP marker reactions:
  1A1/2 (7-ethoxyresorufin O-deethylation)
  2A6 (coumarin 7-hydroxylation)
  2B6 (S-mephenytoin N-demethylation)
  2C8 (paclitaxel 6a-hydroxylation)
  2C9 (diclofenac 4'-hydroxylation)
  2C19 (S-mephenytoin 4'-hydroxylation)
  2D6 (bufuralol-hydroxylation)
  2E1 (chloroxazone 6-hydroxylation)
  3A4/5 (testosterone 6ß-hydroxylation)
  4A11 (lauric acid-hydroxylation)
  11B1 (11-deoxycortisol 11ß-hydroxylation)
  11B2 (deoxycorticosterone –hydroxylation)
- positive controls: CYP reference inhibitors:
  1A1/2 (furafylline)
  2A6 (8-methoxypsoralene)
  2B6 (triethylenethiophosphoramide)
  2C8 (ketoconazole)
  2C9 (sulfaphenazole)
  2C19 (omeprazole)
  2D6 (quinidine)
  2E1 (diethyldithiocarbamate)
  3A4/5 (ketoconazole)
  4A11 (10-(imidazolyl)-decanoic acid)
  11B1 (metyrapone)
  11B2 (ketoconazole)

Analytics

- detection of metabolite formed in the marker reaction

Please inquire for further phase I and II enzyme marker reactions and reference inhibitors.

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2.7. CYP Induction

The induction of CYP isoenzymes is one of the key aspects in drug-drug interaction.
The induction of CYP isoenzymes by the test item is investigated using plated cryopreserved or freshly isolated human primary hepatocytes. The relative fold induction compared to the solvent control is calculated on the level of mRNA, protein, and/or enzymatic activity and compared with that of known reference inducers.

Quantity of test item required: 10 mg
Turnaround time of draft report: 10 – 40 working

Study Design

- cytotoxicity pre-test (optional)
- triplicates
- 48 h recovery phase
- 24 to 72 h induction phase
- one concentration of primary hepatocytes (freshly isolated or cryopreserved)
- one to three concentrations of test item
- negative control: solvent control
- positive control: CYP reference inducers:
  1A2 (omeprazole)
  2A6 (pyrazole)
  2B6 (phenobarbital, rifampicin)
  2C9 (phenobarbital)
  2C19 (phenobarbital, rifampicin)
  2E1 (ethanol)
  3A4 (rifampicin)

Analytics

- enzymatic activity (CYP marker reactions)
- mRNA level (real-time PCR)
- CYP protein content (Western blotting)

Please inquire for further phase I and II enzyme marker reactions and reference inducers.

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2.1. General Considerations

2.1.1. Animal Welfare

It is the overall goal and dedication of GenPharmTox and its founders to replace, reduce, and refine animal experiments whenever possible.

Accordingly, GenPharmTox offers a broad range of well established and cutting-edge innovative *in vitro* assays for preclinical drug development as well as safety evaluation of chemicals. By the many successful projects performed with our clients we have built an exceptionally strong reputation in the area of *in vitro* testing.

The *in vivo* studies offered in co-operation with partners are planned and performed on the basis of sound science and in accordance with animal welfare legislation as well as other relevant regulatory requirements, e. g. OECD- and FDA-guidelines.

2.1.2. Homogeneity and Stability

For each substance tested in *in vivo* studies we recommend performing homogeneity and stability testing including analytical method validation / evaluation in the vehicle used.

2.2. Pharmacokinetics

Currently, GenPharmTox is establishing a new co-operation to provide *in vivo* pharmacokinetic studies.

Please inquire for further information.

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