oroxcell
biopharmaceutical
partner
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industries

Hepatic & Extra-hepatic Metabolism

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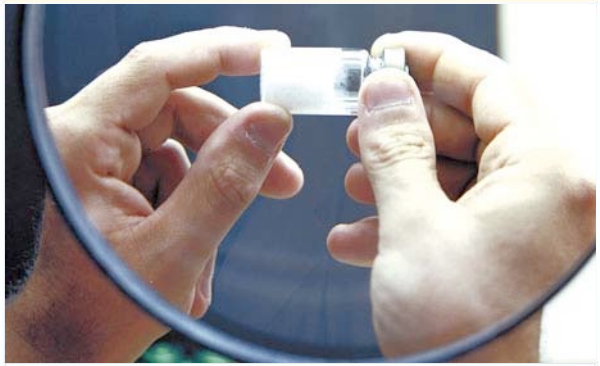
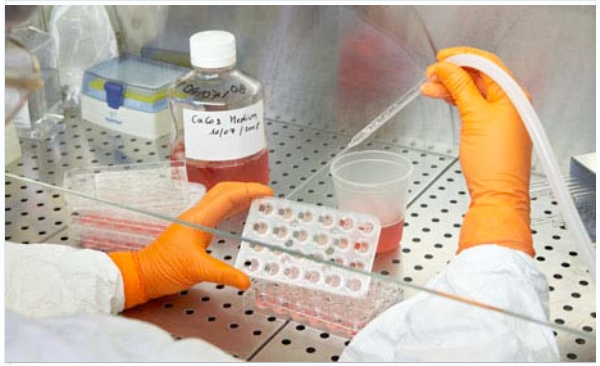




Metabolic Clearance

Information delivered

- Parent compound decline and/or metabolite appearance monitoring
- Intrinsic clearance, half-life and AUC determination
- Interspecies comparisons

Test systems

- Different species hepatic materials: human, rat, mouse, dog, monkey…
- Hepatic material: Individual CYP isoforms, S9, microsomes, hepatocytes
- Extra-hepatic material: skin and intestine microsomes, blood and plasma

Assays

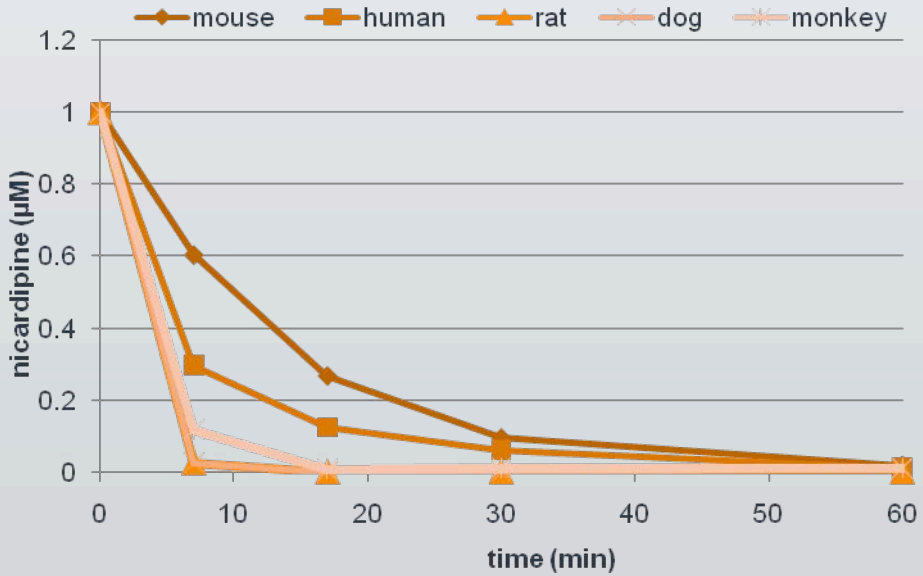
- End-point or kinetic time curves
- Standard or customized protocols
- LC-MS/MS analyses: routine (LC-MS with IS), high quality bioanalysis (Calibration curves/QC), GLP standards under request

Example

Exemple of intrinsic clearance determination

Interspecies intrinsic clearance comparison

- Liver S9 fractions
- Nicardipine 1 µM
- Detection method LC-MS/MS



	$T_{1/2}$ (min)	Cl_{int} (µl.min^{-1}.mg protein^{-1})
Human	34	21
Rat	11	61
Mouse	9	77
Dog	16	43
Monkey	24	29

Metabolic profile determination

Information delivered

- Putative metabolites identified
- Suggested pathways involved in hepatic biotransformations
- Interspecies variation in metabolic profiles (qualitative or quantitative)

Test systems

- Multi-species: human, rat, mouse, dog, monkey, ...
- *In vitro*: S9, microsomes, hepatocytes, Intestinal microflora
- *In vivo*, in rodents, minipigs and dogs

Assays

- Incubation with the test system (standard or customized protocols)
- LC-MS/MS scan search for putative metabolites
- LC-MS/MS in tandem with UV detection and radiodetection
- Quantitative analysis when either metabolite standards or radiolabelled drug are available

In vitro CYP Inhibition

Information delivered

- Potential inhibition of cytochrome P450 activities by the test compound
- Determinations of IC_{50} or K_i for the 5 major human hepatic CYP isoforms:1A2, 2C9, 2C19, 2D6, 3A4

Test systems

- Individual human cDNA expressed CYP isoforms
- Liver microsomes

Assays

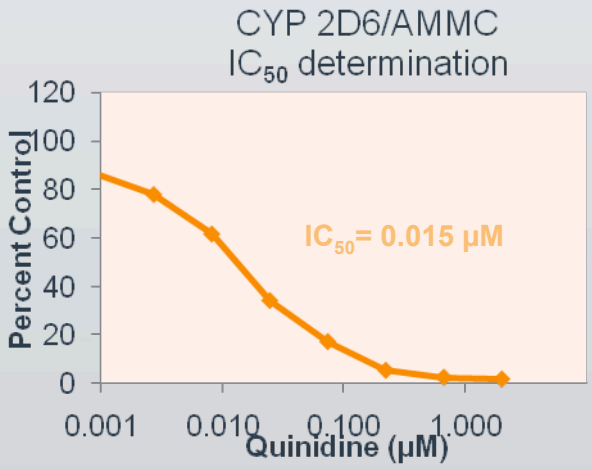
- Validated following FDA guidance for Industry on Drug Interaction Studies
- Recimbinant CYP inhibition assay:
  - Fluorogenic CYP reference substrates
- Microsome CYP inhibition assay:
  - CYP reference substrates
  - LC-MS/MS or UV detection

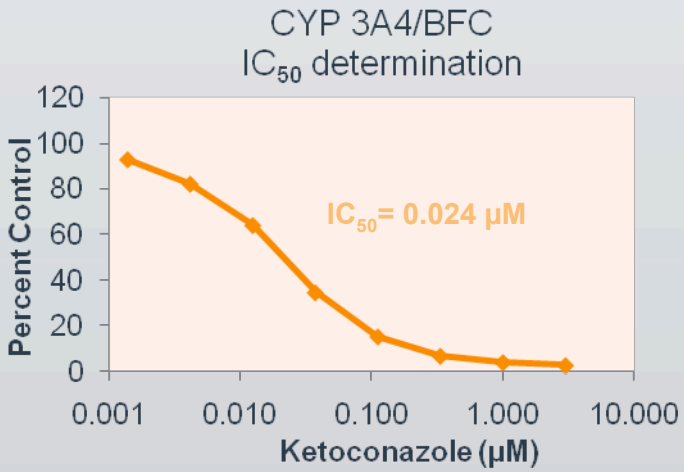
Example

Exemple of IC_{50} determination

Recombinant CYP inhibition assay using fluorogenic substrates

CYP	Substrate	Substrate Name
1A2	CEC	3-cyano-7-ethoxycoumarin
2C9	OOMR	Vivid® CYP450
2C19	BFC	7-benzyloxy-4-trifluoromethyl –coumarin
2D6	AMMC	3-[2-(N,N-Diethyl-N-methylammonium)ethyl]-7-methoxy-4-methylcoumarin
3A4	BFC	7-benzyloxy-4-trifluoromethyl –coumarin
3A4	BQ	Benzyloxyquinoline





CYP-based drug-drug interactions

Enzyme mapping

Information delivered

- Individual CYP P450 isoform(s) involved in drug biotransformation

Test systems

- Individual human cDNA expressed CYP isoforms
- Human liver microsomes
- Hepatocytes

Assays

- Validated following FDA guidance for Industry on Drug Interaction Studies
- Incubation in the presence/absence of specific cytochrome P450 inhibitors
- Standard or customized protocols
- LC-MS/MS analyses: routine (LC-MS with IS), high quality bioanalysis (Calibration curves/QC), GLP standards under request

In vitro CYP induction

Information delivered

- Potential induction of cytochrome P450 activities by the test substance

Test systems

- Freshly isolated or cryopreserved human hepatocytes

Assays

- Validated following FDA guidance for Industry on Drug Interaction Studies

Tests	Exposition duration : over 72 H	Activities measurement at 72h using cytochrome P450 specific substrates
Control	vehicle	• Phenacetine for CYP1A2 • Tolbutamide for CYP2C9 • Testosterone for CYP3A • S-mephenytoine for CYP2C19
Test compound	Range of 5 concentrations	
Reference inducers	• Omeprazole 70 µM for CYP1A2 • Rifampicin 10 µM for CYP2C9, 3A4, 2C19	

- LC-MS/MS analyses:
  - High quality bioanalysis (Calibration curves/QC)
  - GLP standards

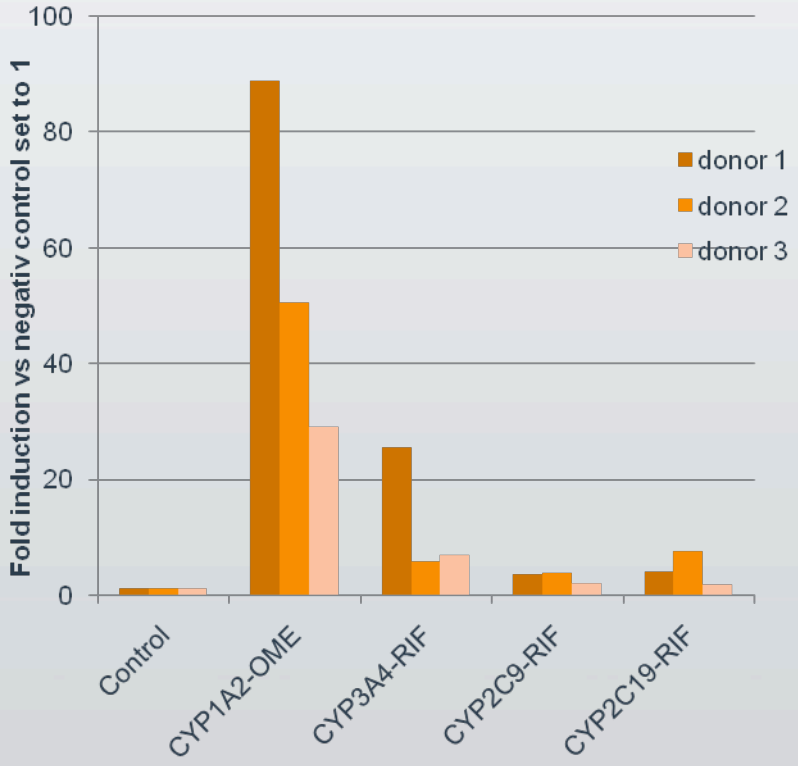
Example

Example of *In vitro* CYP induction

In vitro CYP induction assay using human hepatocytes

In each experiment, the following reference inducers are tested to validate the assay:

- Rifampicin (RIF) for CYP3A, 2C9, 2C19
- Omeprazole (OME) for CYP1A



CYP induction after repeated dosing

Information delivered

Potential induction on cytochrome P450 enzyme activities by the test substance using liver samples from animal species such as mice, rats, dogs or monkeys after repeated dose treatment in toxicology studies

Test systems

Liver sample provided at the end of a repeated dose study

Assays

- Liver microsomes preparation
- Total protein measurement
- Cytochrome P450 activities measured using CYP reference substrates

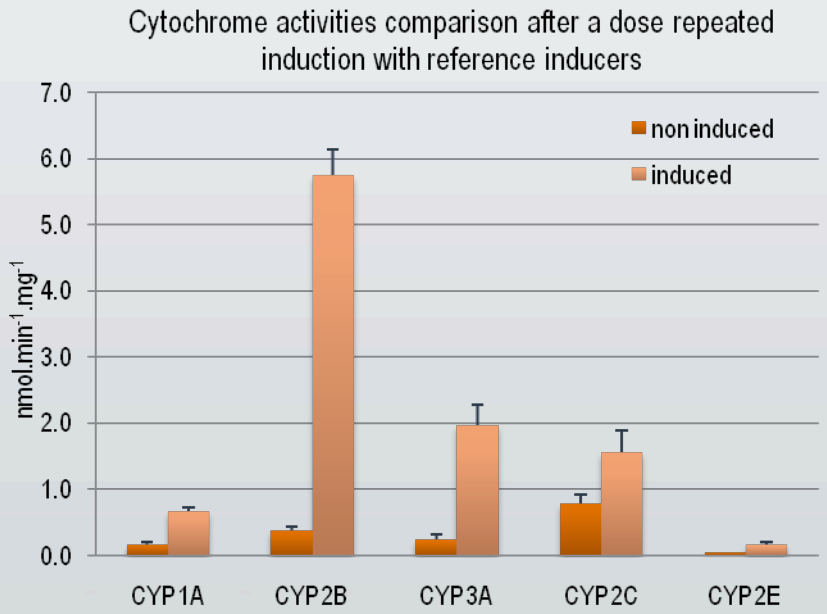
Example

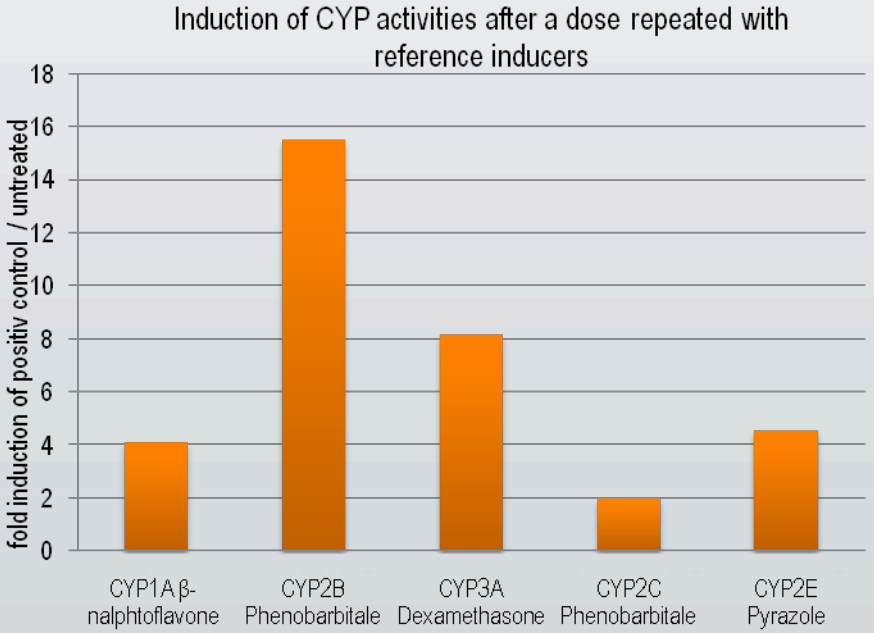
CYP family	Activity measurement	Substrate	Metabolite	Detection method
CYP1A	7-ethoxyresorufin O-deethylase (EROD)	Ethoxyresorufin	resorufin	Fluorimetry
CYP2B	7-Pentoxyresorufin O-dealkylase (PROD)	Pentoxyresorufin	resorufin	Fluorimetry
CYP2C	Aminopyrine N-demethylase (AMD)	Aminopyrine	formaldehyde	Fluorimetry
CYP3A	Erythromycin N-demethylase (ERD)	Erythromycine	formaldehyde	Fluorimetry
CYP2E	Aniline hydroxylase (AH)	Aniline	Para-aminophenol	Fluorimetry

CYP-based drug-drug interactions

Example of CYP induction after repeated dosing

Determination of cytochrome activities on 3 different liver samples using fluorogenic substrates





Extra hepatic metabolism

Stability of the compound in human gastrointestinal contents and colonic microflora

Collaboration with a French hospital

- Healthy volunteers recruitment
- Informed consent of healthy volunteers
- Experiments are conducted following ethics charter

Gastrointestinal tract metabolism

- Intubation of healthy volunteers for collection of GI aspirates
- Fasted and fed conditions
- LC-MS/MS analyses

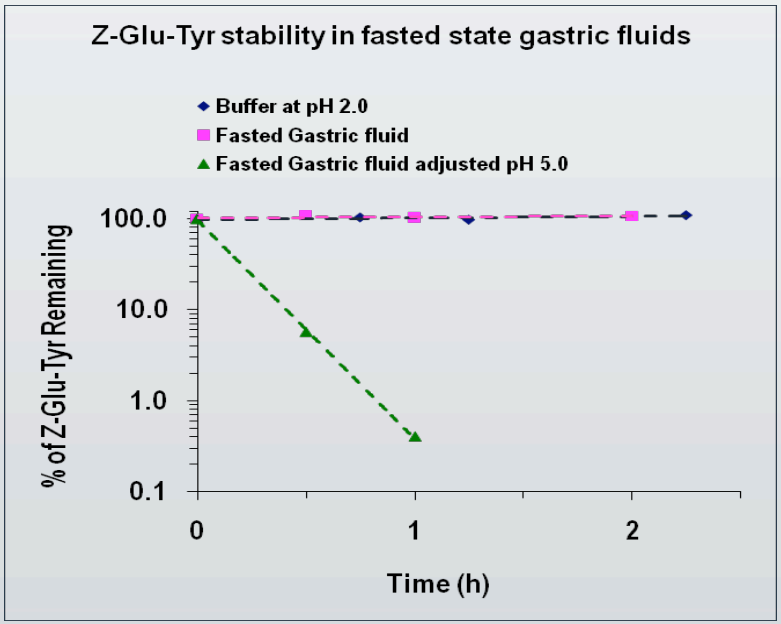
Colonic microflora metabolism

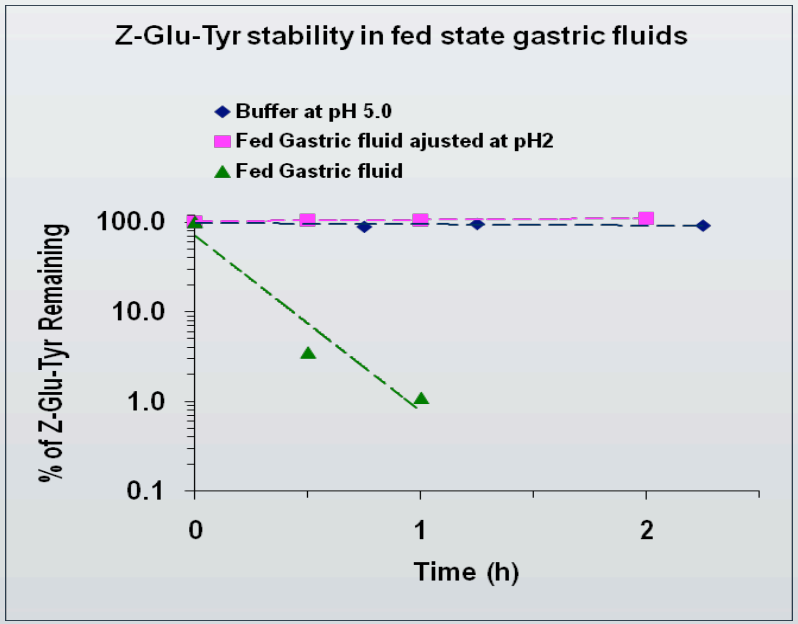
- Anaerobic and aerobic metabolism in colonic microflora
- LC-MS/MS analyses

Examples

Example of human gastric metabolism

Stability of Z-GLU-TYR in human gastric fluids: fasted and fed states



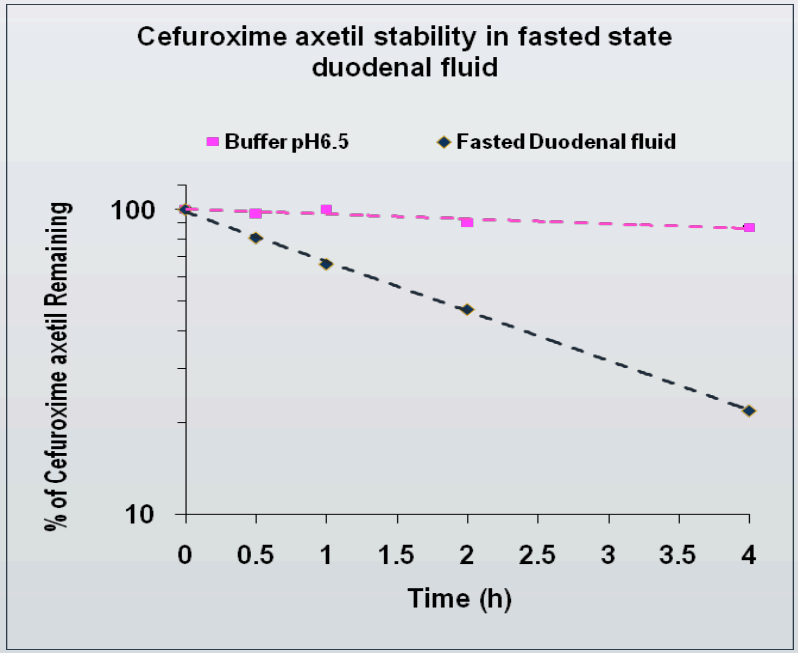


- Chemically stable at pH 2.0
- Stable in fasted gastric fluids
- Instable in fasted gastric fluids buffered at pH 5.0

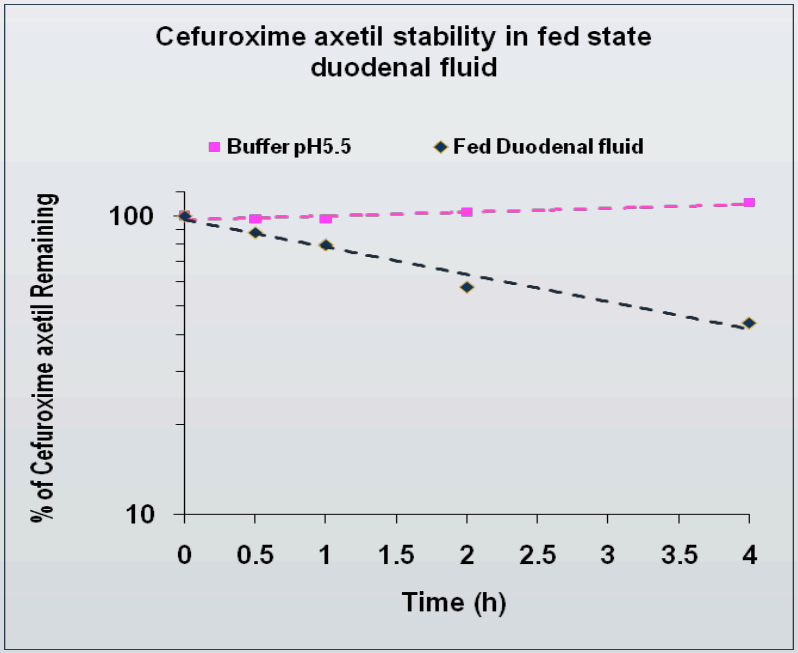
- Chemically stable at pH 5.0
- Stable in fed gastric fluids buffered at pH 2.0
- Instable in fed gastric fluids

Example of human intestinal metabolism

Stability of cefuroxime axetil in human intestinal fluids: fasted and fed states



- Chemically stable at pH 6.5
- Instable in fasted human duodenal fluids
- $T_{1/2}$= 1.8 h

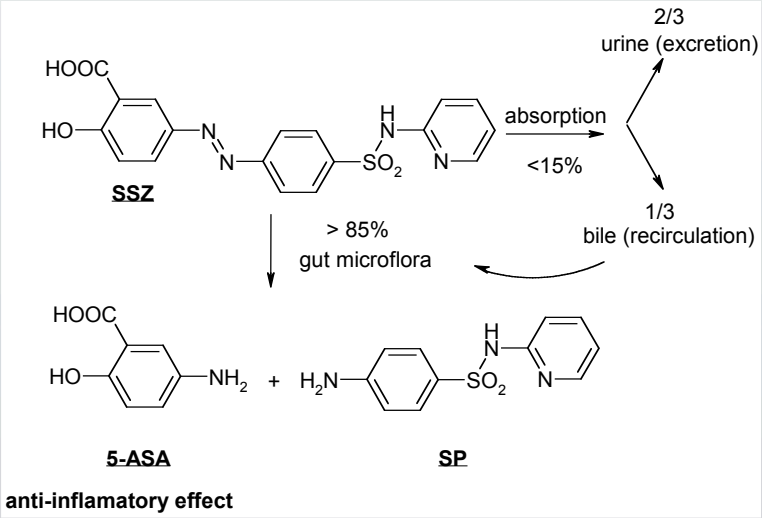


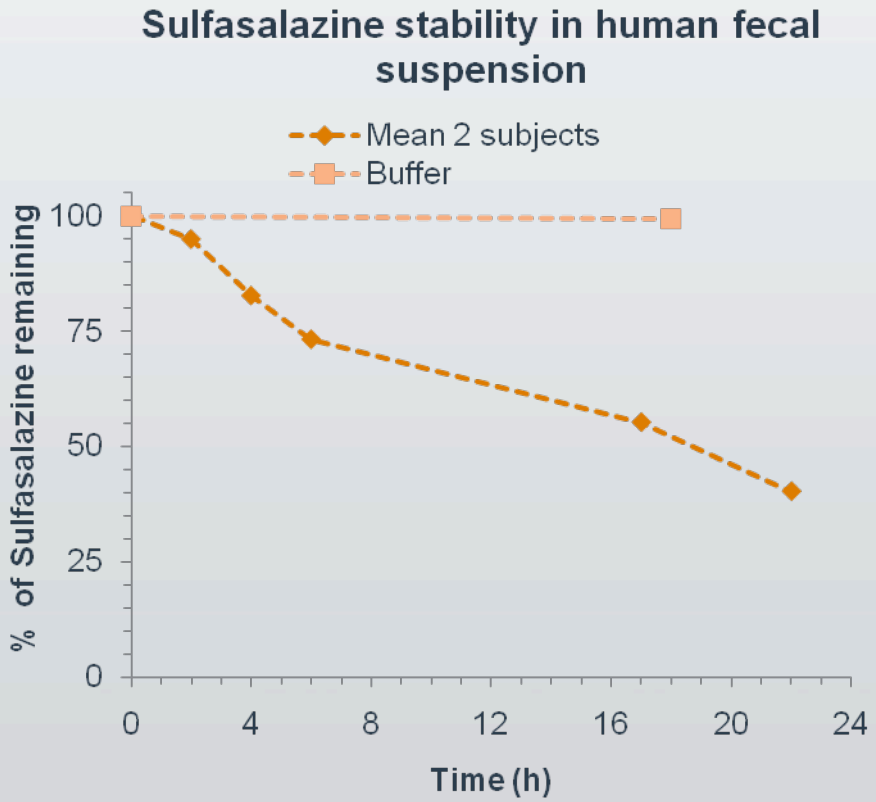
- Chemically stable at pH 5.5
- Instable in fed human duodenal fluids
- $T_{1/2}$= 3.3 h

Example of human colonic microflora metabolism

Sulfasalazine

- Indication: colitis
- Poorly absorbed (<15%)
- Gut microflora metabolism (reductase)
- Not strain-specific
- Generates the active metabolite 5-ASA





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