

ADMET AND ZEBRAFISH SCREENING

OUR CAPABILITIES, SKILLS AND EXPERTISE

ADMET PLATFORM

- State-of-the-art LCMS/MS & NMR instrumentation including: Waters premier coupled to Acquity UPLC, Agilent 6410B coupled to HP 1200 LC, Sciex ABI 4000 and Waters QTOF
- Automated assay process utilising Tecan Genesis and Perkin Elmer Janus®

ABSORPTION ASSAYS INCLUDING

- Aqueous solubility and logP
- PAMPA and Caco-2 permeability A-to-B/B-to-A + P-gp
- MDCK-mdr1

DISTRIBUTION ASSAYS INCLUDING

- Plasma protein binding
- Affinity to HSA (K_d determination)
- Determination of brain to plasma ratio

METABOLISM ASSAYS INCLUDING

- Microsomes, hepatocytes, and plasma stability
- Simulated intestinal & gastric stability
- *In vitro* metabolites identification
- Cytochrome P450 inhibition and induction

TOXICITY ASSAYS INCLUDING:

- HepG2
- Primary rat hepatocytes
- hERG (functional assays and electro-physiology)

PHARMACOKINETICS

- Comprehensive *in vivo* capabilities covering all standard pharmacokinetic parameters, using either non-compartmental or compartmental model for data analysis
- Provision of absorption (K_a) and elimination (K_e) rate constants as well as CL_{tot} , V_{ss} , $t_{1/2}$, AUC and bioavailability

ZEBRAFISH PLATFORM TOXICITY ASSESSMENT

- Hepatotoxicity Screening predicts liver toxicity by assessing morphological changes to the liver
- Developmental Toxicity Screening detects liability for causing embryotoxicity and teratogenicity
- Acute Toxicity Screening predicts toxicity of compounds and determines maximum tolerated concentration (MTC)
- Otototoxicity Screening identifies compounds which may cause hearing loss after acute or chronic exposure

Evotec integrates a comprehensive portfolio of *in vitro* and *in vivo* ADMET assays designed to assist in the optimisation of compounds' pharmacokinetic (PK) properties.

In addition, Evotec's world leading Zebrafish screening platform allows for rapid and cost efficient *in vivo* toxicity and safety pharmacology testing. A range of Zebrafish efficacy models is also available covering major therapeutic areas.

Evotec's Zebrafish platform has the ability to reduce costs by prioritising and profiling compounds in appropriate toxicity assays thus identifying toxicity related issues early in drug discovery programmes.

ZEBRAFISH PLATFORM: SAFETY ASSESSMENT

- Cardiac function assay assesses liability for arrhythmias (bradycardia or tachycardia) and hERG channel blocking
- Locomotor/Seizure assay tests for neurotoxicity and seizure potential
- Bone and cartilage assay test liability for bone demineralisation or loss and cartilage abnormalities

ZEBRAFISH PLATFORM: EFFICACY MODELS

- Evotec has a number of validated efficacy models including: PTZ induced seizure, alcohol induced liver steatosis and chemically induced hearing loss
- Evotec also has the expertise to develop bespoke disease models for customers, using targeted gene manipulation

BIOANALYSIS IN ZEBRAFISH

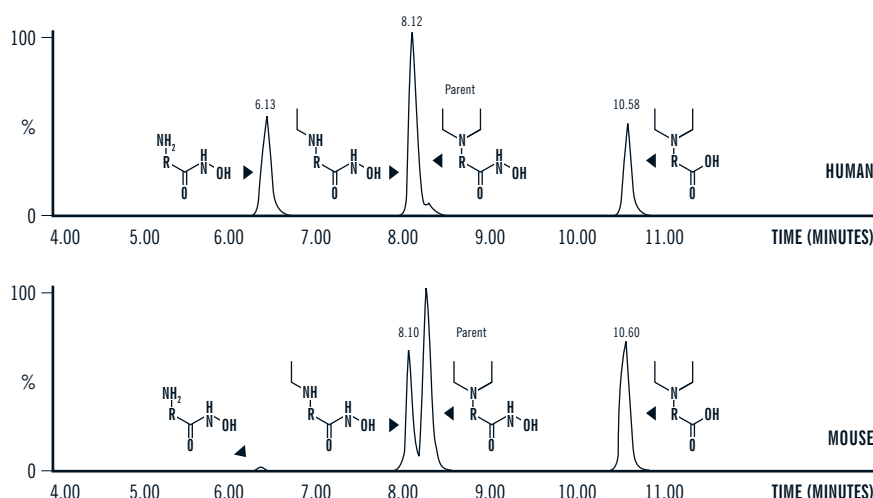
- Bioanalysis is routinely used to measure compound uptake in Zebrafish larvae. This is essential to determine accurate level of exposure and thus minimise false negatives resulting from poor compound uptake

Case studies: Examples of high quality information provided by an experienced team of scientists

METABOLITE IDENTIFICATION

— *In vitro* metabolism identification of drug substance in human and mouse liver microsome using QToF with MS/MS capabilities and accurate mass detection

— Chromatograms showing microsomal metabolism of test compound and conversion of the hydroxamic acid to the carboxylic acid and N-dealkylation at the N-diethylamino group

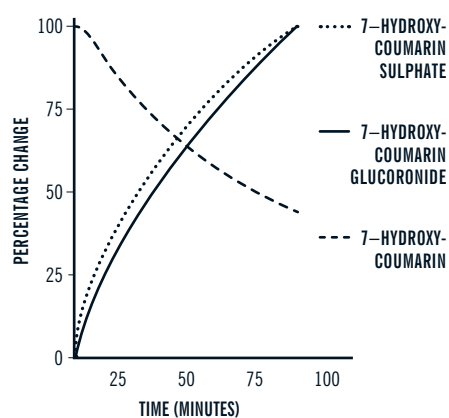


HEPATOCTYTE STABILITY

— Early profiling of compounds for hepatocyte stability provides information on phase 1 and 2 metabolism in the liver

— Mouse hepatocyte stability assay for 7-hydroxy-coumarin and formation of 7-hydroxy-coumarin glucuronide and sulphate

METABOLISM OF 7-HYDROXYCOUMARIN BY MOUSE HEPATOCYTES



Case study: Developmental toxicity and the predictive power of Evotec's leading Zebrafish screening platform

— Evotec led a consortium comprising of 3 major pharmaceutical Companies to evaluate 12 compounds for teratogenicity

— Study demonstrated good correlation between the Zebrafish and *in vivo* data

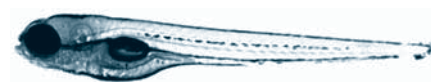
— Overall predictivity = 92%

— For further details on this project, please read: *Steger-Hartmann et al, (2007) Toxicological Sciences 96 (S-1), 94, #450*

Compound	Zebrafish LOEC* (µM)	Effect in Zebrafish	Effect in Mammals
Trichostatin A	0.05	Teratogenic	Teratogenic
Retinoic acid	5	Teratogenic	Teratogenic
Sodium dodecylsulphate**	25	Teratogenic	Non Toxic
Nitrofen	25	Teratogenic	Teratogenic
Mometasone furoate	5	Teratogenic	Teratogenic
Carbaryl	25	Teratogenic	Teratogenic
Y27632 (rho kinase inhibitor)	500	Teratogenic	Teratogenic
Cladribine	500	Teratogenic	Teratogenic
Lithium Chloride	300,000	Teratogenic	Teratogenic
Benzoic acid	>500	Non Toxic	Non Toxic
Captopril	>500	Non Toxic	Non Toxic
Chlorzoxazone	>500	Non Toxic	Non Toxic

* LOEC = Lowest Observed Effect Concentration

** Sodium dodecylsulphate is a known toxicant to fish in general



Control larva



Treated larva