Pharmacelsus®

CONTRACT RESEARCH ORGANISATION

GLP CERTIFIED

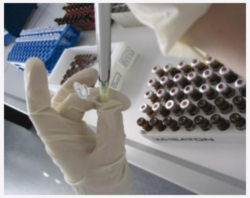
Research is more than our business, we love it !

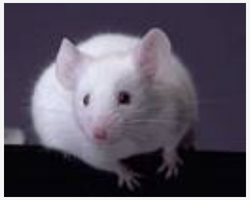
OUR EXPERTISE

- Founded 2000
- 9 state-of the-art laboratories, GLP-certified since 2008
- Service Portfolio: ~100 assays
- 99 % projects delivered, 95 % repeat business

Areas of Expertise:

- Drug Discovery Services
- Early Drug Development Services
- Bioanalytical Services - G(C)LP and non-GLP
- Bioanalytical Method Development






Why are toxicity biomarkers important?

1st Goal of Drug (Discovery) research: to identify new compound with defined pharmacological effect

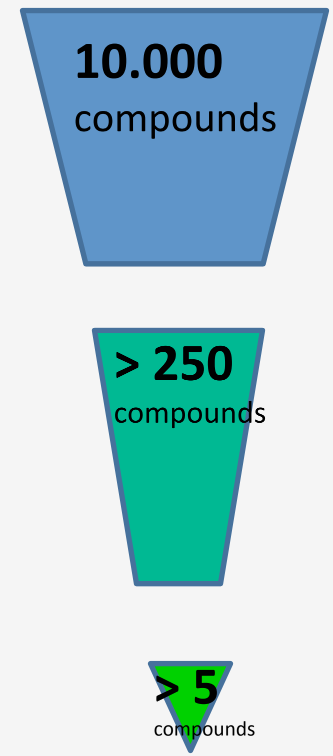
-> discover high number of new potential drugs

2nd Goal of Drug (Development) research: to ensure safety and efficacy and discard unpromising compounds

-> reduce the number of compounds

Identifying problems early on saves money.

Identification and implementation of predictive early biomarkers is necessary.



Why is finding new biomarkers for liver toxicity important?

(1) Hepatotoxicity is still a major reason of safety-related drug withdrawals. From 1975-2007, 150 drug candidates had to be withdrawn – about 30 % due to hepatoxicity issues (Hornberg et al., 2014).

(2) Serum enzymatic activity of alanine aminotransferase (ALT) is considered the gold standard clinical chemistry biomarker of liver injury in both preclinical species and humans (Ozer et al., 2010). BUT: increases in ALT activity don't always correlate with histopathology findings (Ennulat et al., 2010).

-> There is a clear need for more predictive biomarkers.

Recent publications indicated that changes in BA profiles were characteristic for toxic response in the liver (Lin et al., 2009; Yamazaki et al., 2013).

-> Bile acids (BAs) are potential biomarkers for liver toxicity.

Bile Acids: Early Biomarkers of Liver Toxicity

Published data: Altered levels of bile acids are linked to specific phenotypes.

Bile Acid	Cytotoxity/ Apoptosis		Bile Duct Hyperplasia		Inflammation	Cholestase
	Human	Rat	Human	Rat		Human
DCA	+	+				-
TLCA	+	+				-
GCDCA	+	+				+
UDCA	-	-				-
TUDCA	-	-				+
CA						
TCA			+	+	+	+
GCA			+	+		+
α + β MCA					+	
TMC					+	

Data taken from: Luo et al, 2014, Woolbright et al., 2012, García-Cañaveras et al., 2012

Bile Acids: Early Biomarkers of Liver Toxicity

Objective: Examine the effect of drug-induced liver injury on bile acid profiles in rats.

Animals: male Sprague Dawley rats (~300 g body weight)

Surgery: catheterisation of V. jugularis/3 days of recovery from surgery

Set-up: test compound administration at t_0
blood sampling and preparation of Li-heparin plasma:
pre-dose and 0.25, 0.5, 1, 2, 4, 8, 24 h post dose
sampling of liver tissue at necropsy, 24 h post dose

Group	Test item	Formulation	Dose	Volume	Route	n
1	Acetaminophen	0.5% MC	1000 mg/kg	5 ml/kg	po	6
2	Diclofenac	0.5% MC	15 mg/kg	5 ml/kg	po	6
3	Ethinylestradiol	olive oil	5 mg/kg	5 ml/kg	sc	6
4	Vehicle	0.5% MC	n.a.	5 ml/kg	po	6

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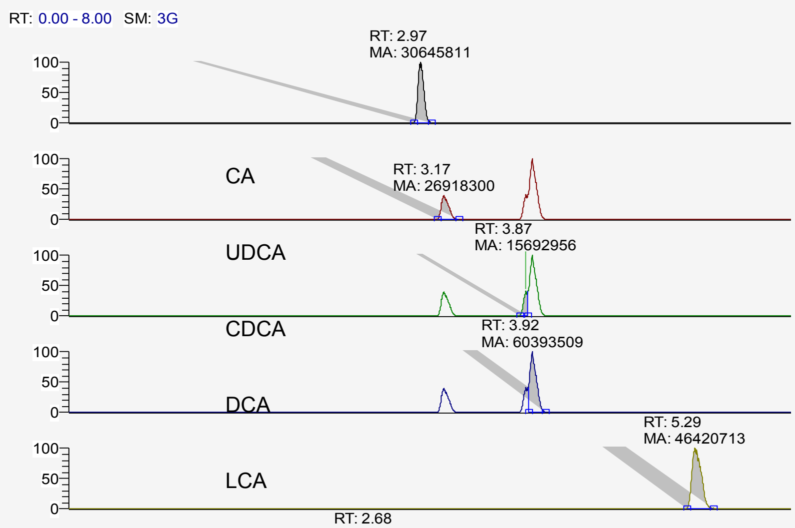
Bile Acids: Early Biomarkers of Liver Toxicity

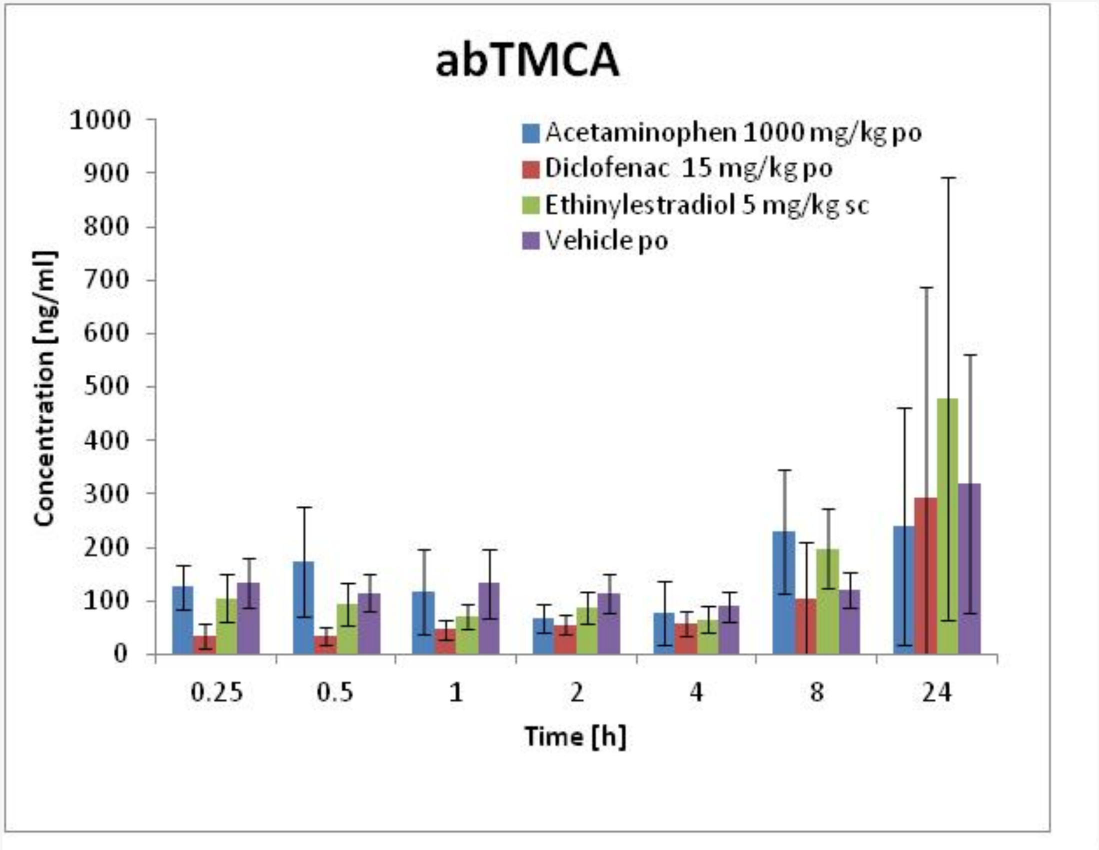
Challenge: Develop a qualified method for HPLC-MS analysis and qualification of 14 bile acids in one sample (cassette analysis)

Quantitative LC-HRMS analysis was performed applying simultaneous dual polarity measurement in a single run (Q-Exactive mass spectrometer with Orbitrap accurate mass technology). Lower limits of detection were found in the range of 0.2 ng/ml to 0.4 ng/ml (UDCA, CDCA, DCA, TUDCA, TDCA, GUDCA, GCDCA, GDCA).* Accuracy and precision were within the acceptance criteria of the FDA/ EMA validation guidelines for quantitative bioanalytical methods.



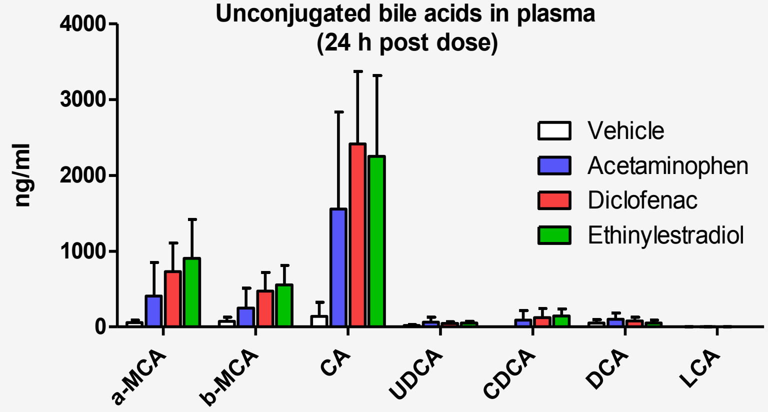
Chromatogram resulting from the simultaneous bile acid analysis.

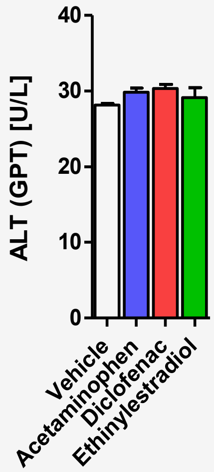
Results: Changes of bile acids over a period of 24 hours

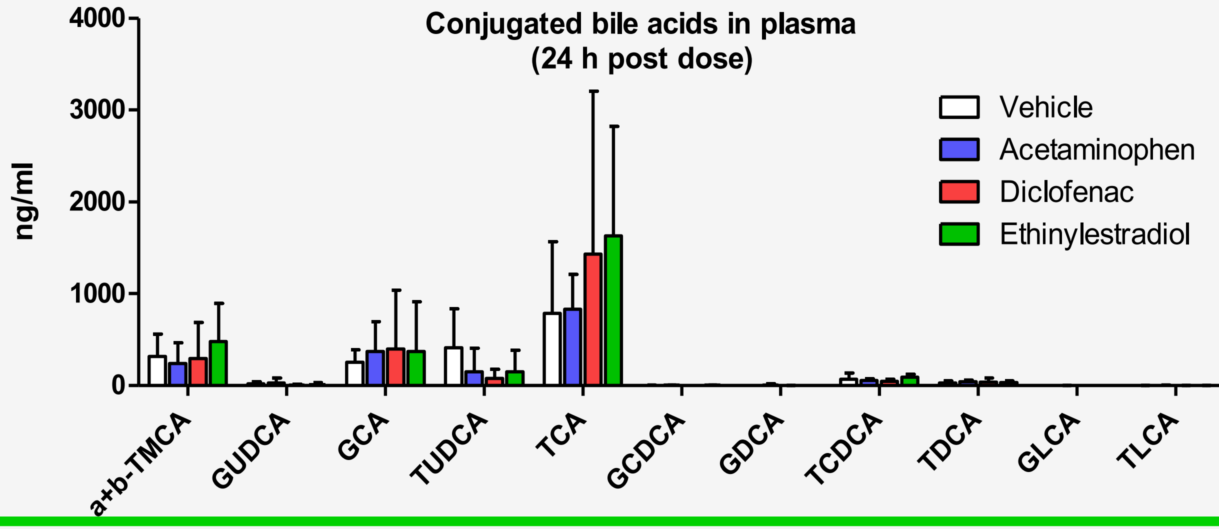


Changes in bile acid plasma levels were observed during 24 h. At 24 hours differences between animals treated with vehicle only and compounds where most prominent.

Results: Means ± SD of plasma bile acid concentrations 24 h post dose

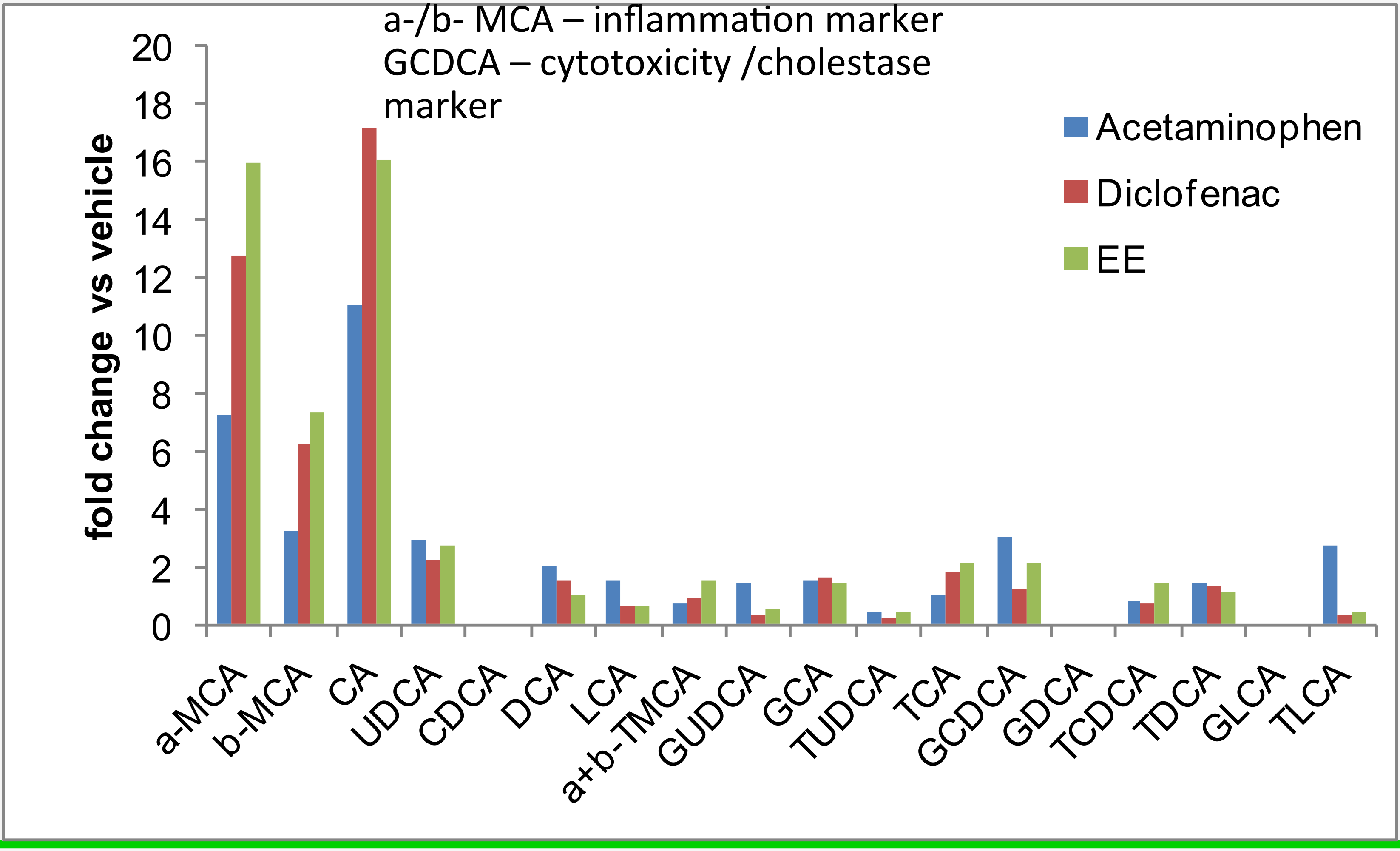






Bile Acids: Early Biomarkers of Liver Toxicity

Results: **Fold change of mean plasma bile acid concentrations 24 h post dose (fold change vs vehicle control)**



Conclusions:

- Sensitive mass spectrometry is able to quantify bile acids.
- After application of compounds known for inflicting liver injury increase of bile acids linked to liver toxicity in the liver could be clearly observed.
- Levels of bile acids change fast: within 24 h – classical *in vivo* toxicity studies take 2 to 6 weeks.
- Different liver toxic compounds result in different alterations in levels of bile acids.

Analysing changes in bile acids at 24 hours after compound application allows for an early assessment of liver toxic tendencies of compounds.

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