Intelligent Drug and Product Profiling: *in vitro* und *in vivo* Teststrategien zur effizienten präklinischen Entwicklung

Across Barriers GmbH
Dr. Udo Bock, *Chief Technical Officer*

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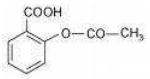
across barriers
Intelligent Drug Profiling

Company profile Across Barriers

- Foundation by Prof. C.-M. Lehr and Dr. E. Haltner in 1998
- 1.000 m² working area
- S1 labs for biological and radioactive operations
- 37 Employees (pharmacists, chemists, biologists)
- Permissions and quality standards
  - **11/00** Permission for radioactive work
  - **07/01** GLP certified, 3 categories (Good Laboratory Praxis),
  - **07/02** test of drugs and drug products; GMP
  - **02/03** Permission to work with Narcotic Agents
  - **10/07** biological safety level S1 (GMOs)
  - independent quality assurance, double check of all data, frequent training for our employees

Product Development Steps

Jahre	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Entwicklungs-phasen	präklinische Entwicklung				klinische Entwicklung							Markteinfüh-rung		Monitoring nach Markteinführung
	Synthese biologische Tests pharmakologisches Screening				Phase I 50 -100 gesunde Probanden		Phase II 200 - 400 Patienten od. erkrankte Probanden	Phase III >3000						Phase IV
					toxikologische and pharmakokinetische Studien									
					Tierstudien									
					chemische Entwicklung									
					pharmazeutische Entwicklung									
Anzahl zu untersuchender Substanzen	> 10,000 (klassisch)				8 -15		4 - 8	2 -3				1 -2		1

Some facts about oral dosage forms.....

- About 50% of the marketed drugs are still **oral** dosage forms e.g.
  - high compliance
  - line extension
  - long stability
  - low production costs
- Estimated cost > 500 million USD or more
- Estimated time up to 12 years (also we have tremendous innovation)

⇒ **High costs/timelines reflect the high rate of failure in early phases of drug development**

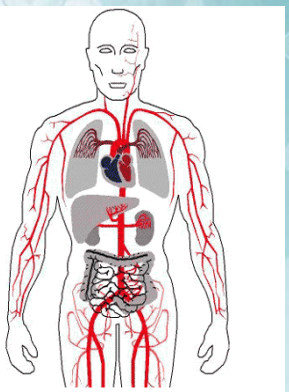
- Attribution of failure
  - 10 % lack of efficacy
  - 5 % commercial reasons
  - **39 % poor pharmacokinetics or inadequate bioequivalence for generics**

⇒ **Major efforts are directed to identify and eliminate compounds that are not likely to have "drug-like" properties or to reach bioequivalence**

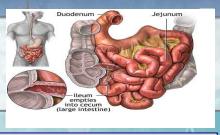
J. Pharm. Sci 93:239-255 (2004); Nature Reviews Drug Discovery 2: 247 (2003)

Outline – Intelligent Drug and Product Profiling

- **Oral Dosage Forms**
  - Relevance of efflux transporters
  - Drug – Drug Interaction Studies
- **Topical Dosage Forms**
  - Skin Permeation with patches
  - NSAIDs *in vitro* and *in vivo* Penetration
- **Pulmonary Delivery**
  - pBCS – pulmonary Biopharmaceutics Classification System
  - Formulation selection
- **Compatibility testing**
- Summary
- Outlook and potential **Collaborations**



Different in vitro Methods to Predict Intestinal Absorption



Discovery

- In silico models
- logP or logD
- pka
- k'
- BBMV
- Solubility
- Pampa
- Caco-2 MDCK-WT MDCK-mdr

Development

- Ussing
- Gut loop or perfusions
- In vivo animal
- Fa % in humans

Golden Standard

Prediction, costs, time, expenditure

Automation, Reproducibility

Factors Influencing Oral Absorption

Formulation factors
Dosage form, absorption enhancers, Coatings, stability, solubility enhancement

Physicochemical factors
Size, solubility, molecular weight, charge (pKa), H-bonding potential, molecular surface area, stability

Tablet

Disintegration

Dissolution

Aggregates

Drug in solution

Drug in blood

Permeability

Biological factors
Stomach emptying time, intestinal motility, lumenal conditions (pH, content, metab. activity), diseases

Fine particels

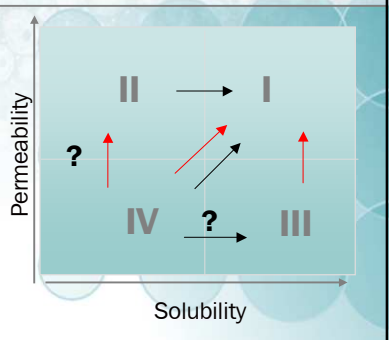
Biochemical factors
Metabolism, efflux, active transporters

Know more about your API
Benefits of biopharmaceutical classification system

I High Solubility – High Permeability
- The behaviour of drug and excipient are independent
- GI permeability is not the limited factor
- Absorption dependent on gastric emptying
- Interaction and stability are the two main points
- IVIVC correlation
- **Conventional formulations**

II Low Solubility – High Permeability
- **Solubility enhancers** should be included **in formulations** for efficient bioequivalency
- IVIVC might be possible

III High Solubility – Low Permeability
- **Permeability enhancers** should be included **in formulations** for efficient bioequivalency
- In some cases IVIVC

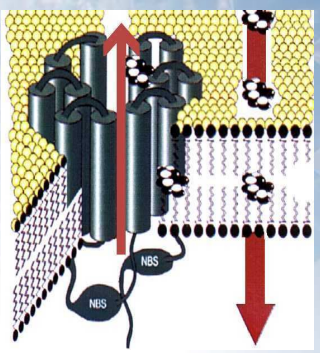


Increase permeability

Increase solubility or solubility rate

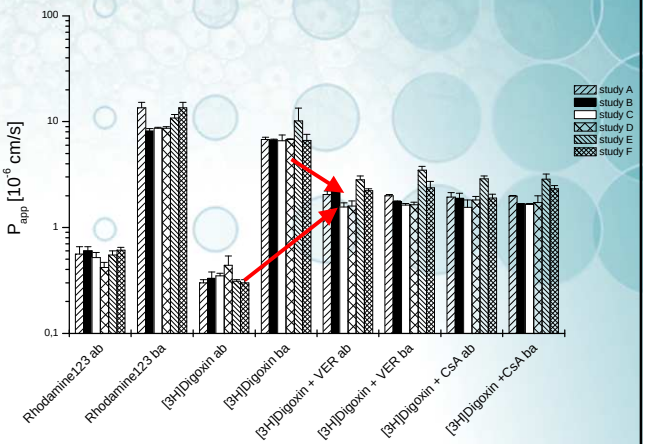
Active Carriers: P-Glycoprotein (P-gp) as example for active transport

- Transmembrane protein
- Active efflux of chemically diverse compounds
- Physiological function: detoxification of cells
- Key role in barrier properties
- **Effects of efflux mediated transport of drug molecules**
  - Species dependent bioavailability
  - Large interspecies variability of absorption due to differing expression of efflux systems
  - Induction of efflux systems resulting in decreasing absorption or faster elimination
  - Interactions with phytopharmaca (e. g. St. John's Worth)
  - Interactions with a wide range of drugs
  - Interactions with food (e. g. herbal tea; fruit juice...)



Pgp-marker Compounds and Inhibition Studies

- **Rhodamine 123**
  ab and ba
- **Digoxin**
  ab and ba
  (Fa: 81%)
- **Inhibitors**
  - Verapamil (VER)
  - Cyclosporin A (CsA)



Guideline on Drug – Drug Interactions

Guidance for Industry

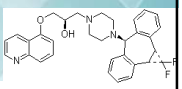
Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling

DRAFT GUIDANCE

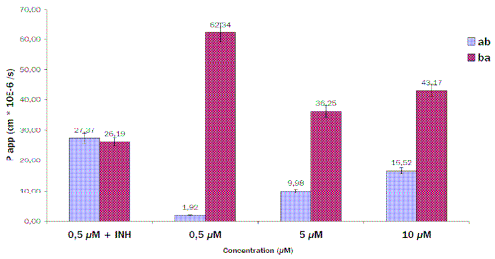
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

September 2006
Clinical Pharmacology

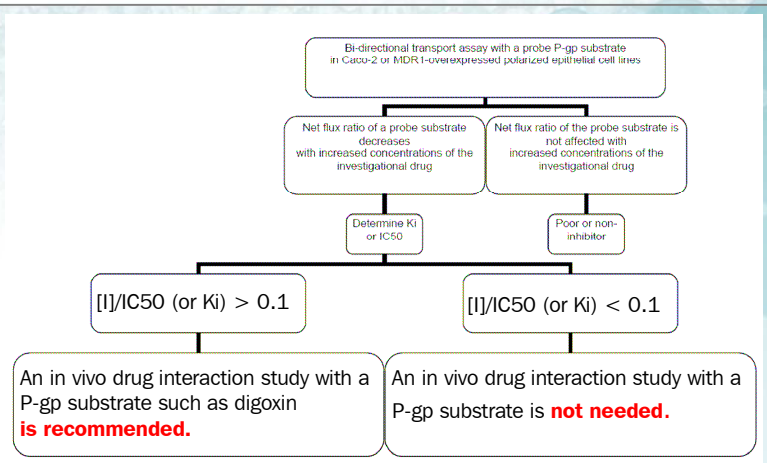
- Quinidine across Caco-2
- LY335979 (Zosuquidar trihydrochloride)
  - Highly specific p-gp inhibitor
  - Ki of 59 nM
  - Full inhibition 150 nM



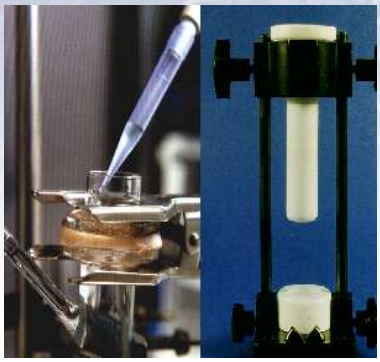
EXAMPLARY SUBSTRATE -QUI- TRANSPORT EXPERIMENT

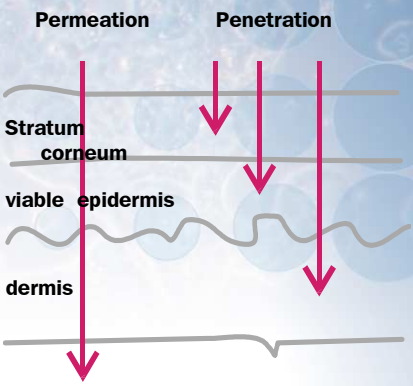


Decision Tree for Drug Interactions



Dermal ex vivo Systems (human und porcine)

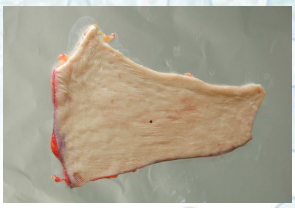


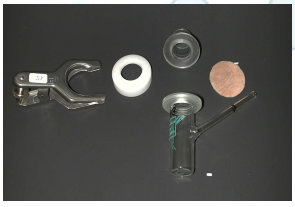


Ansicht der vorherigen Seite ...

Skin permeation in vitro

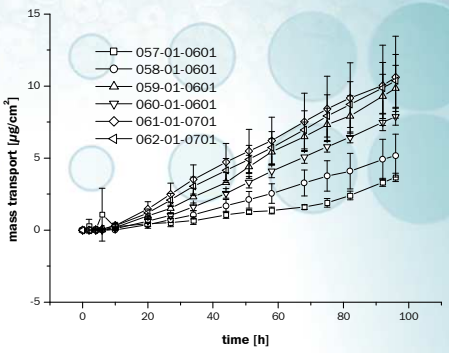
- Human skin (full thickness, dermatomized skin, heat-separated skin or trypsin isolated SC)
- 36mm punch
- Transferred into Franz diffusion cells (20 mL acceptor volume, 3 cm^2 skin surface)
- Assembling and performance at 32°C





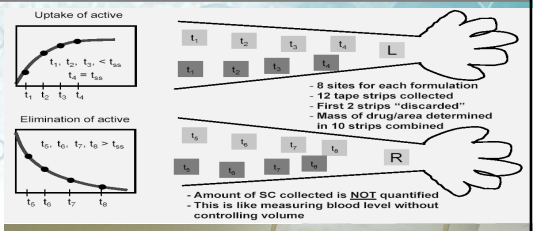
Permeation of estradiole from matrix TTS across human skin in vitro

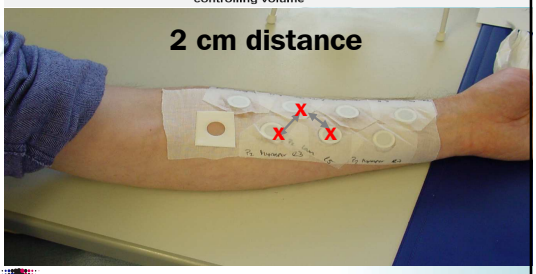
- Dermatomized human skin (500 μm)
- 96 hours, n=3, 32 °C
- 6 individuals
- Age from 40 to 60 years
- Female
- Knowledge of SC thickness
- Knowlegde of caffeine permeabiltiy
- Documented skin color
- Performed according to SOP M 005 Permeation in vitro



In vivo Penetration in accordance to FDA Guideline

- FDA Guidance "Topical Dermatological Drug Product NDAs and ANDAs – In vivo Bioavailability, Bioequivalence, In vitro Release, and Associated Studies", June 1998
- Test formulation
- Left and right arms
- finite dose
- 2-3 mg/cm²
- Control(s)
- Kinetics
- up to 8 positions per arm





Ketoprofen 1/2 - Bock et al. Arzneim-Forsch./Drug Res. 54, No. 9, 522-529 (2004)

- **In vitro [µg/cm²]**
  - 3 h incubation
  - Stratum Corneum (SC) 37.7 ± 12.1
  - Deep Skin 27.3 ± 8.5

Tab. 3: Transportierte Masse pro Fläche [µg/cm²] von Ketoprofen aus Test-D und Ibuprofen aus Ref-D in exzidierte Humanhaut (Nr. 086-01-0202, 089-01-0302 und 091-01-0402).

Wirkstoff	Formulierung	Haut Nr.	Gehalt	SC [µg/cm²] cm²]	DS [µg/cm²] cm²]
Ketoprofen	Test-D	086-01-0202	2,5 %	5,6	7,2
		089-01-0302	2,5 %	1,6	1,1
		091-01-0402	2,5 %	4,2	7,3
Mittel	Test-D	–	2,5 %	37,7 ± 12,1 12,1	27,3 ± 8,5 8,5
Ibuprofen	Ref-D	086-01-0202	5,0 %	23,3 ± 2,7	38,1 ± 2,6
		089-01-0302	5,0 %	29,1 ± 7,3	69,1 ± 12,4
		091-01-0402	5,0 %	37,9 ± 13,0	113,9 ± 13,4
Mittel	Ref-D	–	5,0 %	30,1 ± 6,0	73,7 ± 31,1

SC = Stratum corneum, DS = tiefere Hautschichten (Mittelwert ± Standardabweichung, n = 3).

- **In vivo [µg/cm²]**
  - 3 h incubation, only SC
  - Person 1: 97.0 ± 14.8
  - Person 2: 81.1 ± 17.8
  - Person 3: 62.2 ± 11.2

Tab. 4: Transportierte Masse pro Fläche [µg/cm²] von Ketoprofen (Gehalt 2,5 %) aus Test-D in humane Haut (Stratum corneum) in vivo (Mittelwert ± Standardabweichung, n = 3).

Applikationsdauer (h)	Person	Transportierte Masse pro Fläche [µg/cm²] im SC
0,5	1	46,9 ± 12,0
1		58,9 ± 12,2
3		97,0 ± 14,8
6		65,6 ± 5,3
0,5	2	38,8 ± 5,8
1		56,0 ± 8,5
3		81,1 ± 17,8
6		61,6 ± 9,3
0,5	3	37,4 ± 6,7
1		41,6 ± 10,3
3		62,2 ± 11,2
6		67,6 ± 17,3

Ketoprofen 2/2 - Bock et al. Arzneim-Forsch./Drug Res. 54, No. 9, 522-529 (2004)

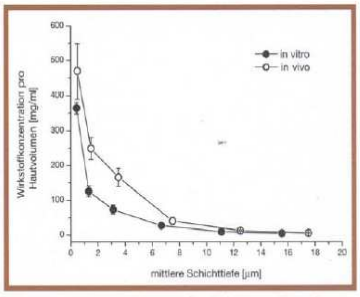


Abb. 5: Penetration von Ketoprofen in humane Haut (Stratum corneum) in vitro (3 Häute) und in vivo (3 Personen) nach 3-stündiger Inkubation mit Test-D. Dargestellt sind die Konzentrationstiefenprofile im Stratum corneum. Die angegebenen Werte sind Mittelwerte ± Standardabweichung von 3 Häuten bzw. 3 Personen an denen die Penetrationsstudien jeweils in Dreifachbestimmung durchgeführt wurden.

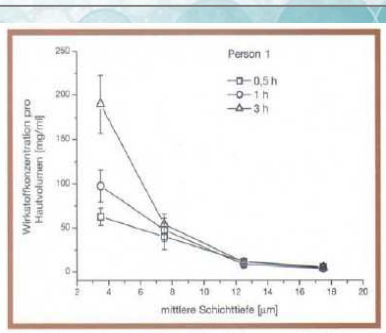
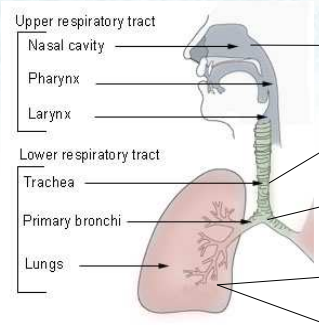


Abb. 4: Penetration von Ketoprofen in humane Haut (Stratum corneum) in vivo (Person 1) nach 0.5- bis 3-stündiger Inkubation mit Test-D. Die ersten beiden Tesastrips wurden hier nicht berücksichtigt. Dargestellt sind die Konzentrationstiefenprofile im Stratum corneum. Die angegebenen Werte sind Mittelwerte von Dreifachbestimmungen ± Standardabweichung.

Aerolized CsA for prevention of organ rejection in lung transplant -

Trammer et al., EJPB, 2008;70(3):758-64



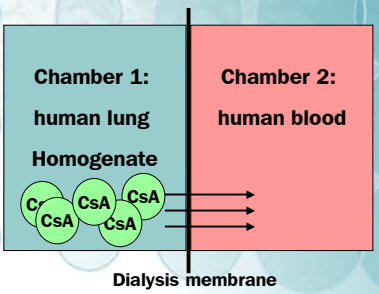
RPMI 2650

9HTE16o-

Calu-3

NHBE

16HBE14o-



Dialysis membrane

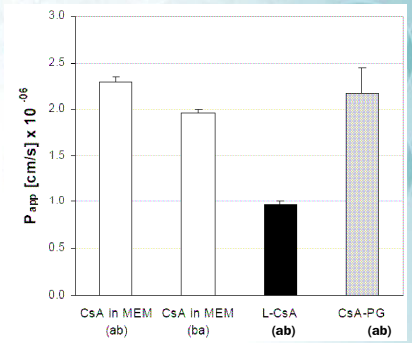
A549 (build no tight monolayers)

Primary cells (porcine)

High yield, anatomically, physiologically and biochemically similar to human

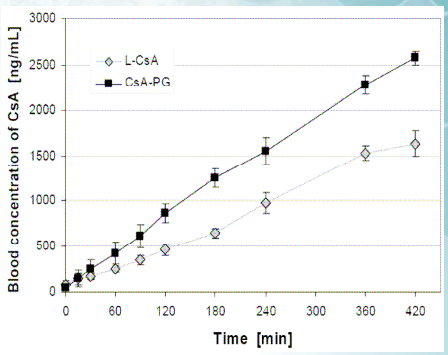
Results at Calu-3

- **Papp of CsA formulations across Calu-3 monolayers (*in vitro* model)**
  - CsA is low permeable
  - CsA transport has no directionality
  - L-CsA shows lower permeability than CsA-PG



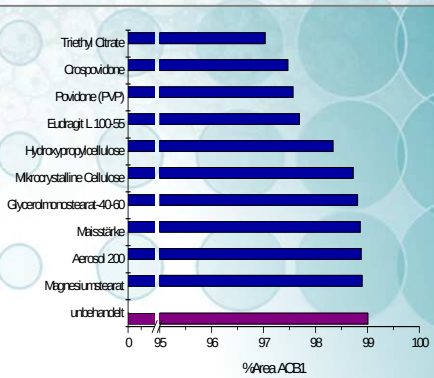
Results at the ex vivo model

- **CsA diffusion towards the blood compartment (*ex vivo* model)**
  - CsA diffusion across the dialysis membrane
  - Area under the curve (AUC) and diffusion rate (DR) determined
  - CsA-PG is absorbed by the blood compartment faster than the liposomal formulation
  - The model did not allow calculation of c_{max}, t_{max} and $t_{1/2}$ (drug transfer was not completed): the model mirrors the absorption phase



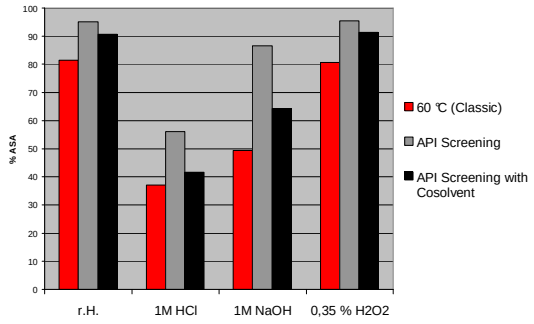
Stress stability and pre-formulation screening for liquid, solid and semisolid formulations

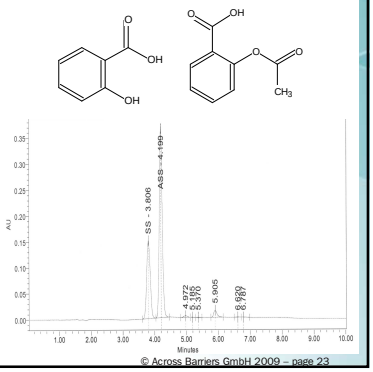
- **Benefit – one mg one day approach**
  - Low sample amounts (approx. 30 mg of API for a set of 20 excipients)
  - Very fast result (usually within 10 working days full report available)
  - Chromatographic Impurity Profile available for each API-excipient mixture
- **Information we get**
  - purity of drug
  - Influence of conditions (acid, base, oxidation)
  - Influence of API/excipient ratio
  - Influence of excipient quality resp. purity
  - Influence of the granulation/tableting process
  - No information about kinetic



- **Two step procedure**
  - Assessment of API stability
  - Assessment of API-excipient interaction

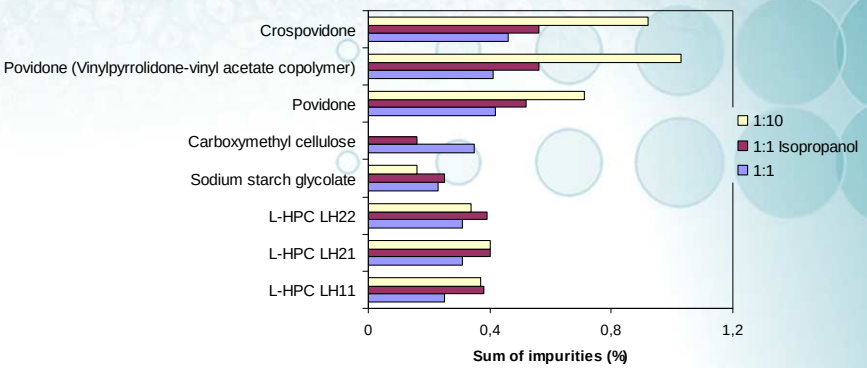
Example of API forced degradation: Acetylsalicylic acid





Compatibility screening of Raloxifene with selected excipients

L-HPCs as preferable excipients identified (instead of commonly used Povidones) to reduce oxidative degradation

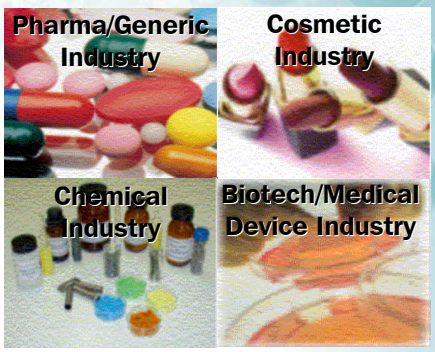


Across Barriers' in vitro ADMET models

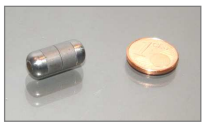
- In vitro permeability (standardized and customized assays)
- Investigation of transport mechanisms
  - identification of active transport mechanisms
  - identification of efflux mediated transport
  - interaction of drug substances
  - influence of excipients on permeability and/or transport mechanism
  - proof of drug delivery concepts
- Dermal Barrier (porcine, human, reconstructed skin)
- Gastrointestinal Barrier (Caco-2, porcine gut)
- Blood Brain Barrier (primary endothelial cells)
- Pulmonal Barrier (bronchial cells, lung cells)
- Buccal and nasal Barrier (excised tissue)
- Human vaginal and cervical barrier (excised tissue)
- Classification of drug substances according Biopharmaceutics classification system (FDA and EMEA)

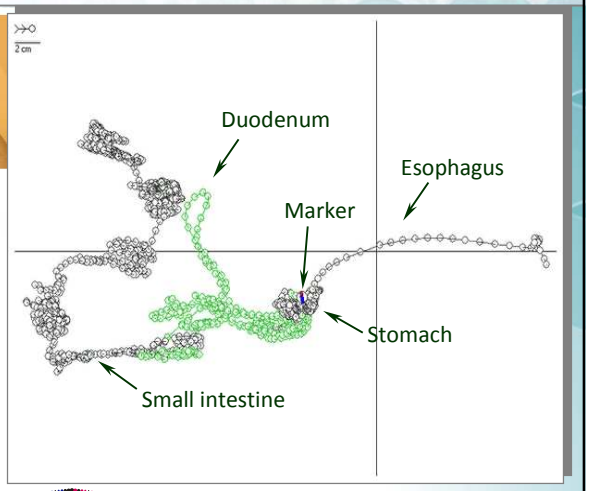
How to work with us

- Fee for Services
- Strategic Alliances
- Consulting
  - support with authorities
  - regulatory support
  - customized study design
  - project planning
  - projectmanagement
- **R&D Cooperations**
- Frame Work Agreements



MAARS - Magnetic Active Agent Release System



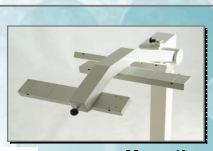


MAARS - Magnetic Active Agent Release System

MAARS Capsule

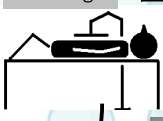
Phase 1 Oral intake

Phase 2 Tracking

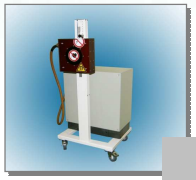


Magnetic tracking

Phase 6 Decision



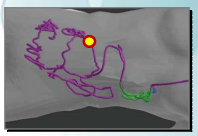
Phase 5 Evaluation



Release process

Phase 4 Plasma concentration

Phase 3 Release



Path of the capsule

Patent : DE 10302614.2-44

The Company | Our Services | Our Techniques | Future Projects

MAARS - Magnetic Active Agent Release System

MAARS – Selection of services

- Testing of drug-drug interactions
- Inhibition studies with efflux transporters (e.g. Pgp, MRP-2, BCRP)
- Side specific absorption windows (e.g. Duodenum, Ileum, Jejunum, Colon)
- Impact of polymorphism, pH value or salt forms on gastrointestinal absorption
- Testing of principle of drug delivery approaches
  - Mucoadhesion
  - Micro- and nanoparticles
  - Enzyme inhibition gastrointestinal absorption
- Selection of solubilizer (BCS II compounds) or Penetration enhancer (BCS III compounds)

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Thank you for your attention!

Dr. Udo Bock
Chief Technical Officer
u.bock@acrossbarriers.de

Science Park 1
66121 Saarbrücken, Germany
Phone: +49 681 959 188 00
Fax: +49 681 959 188 02

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