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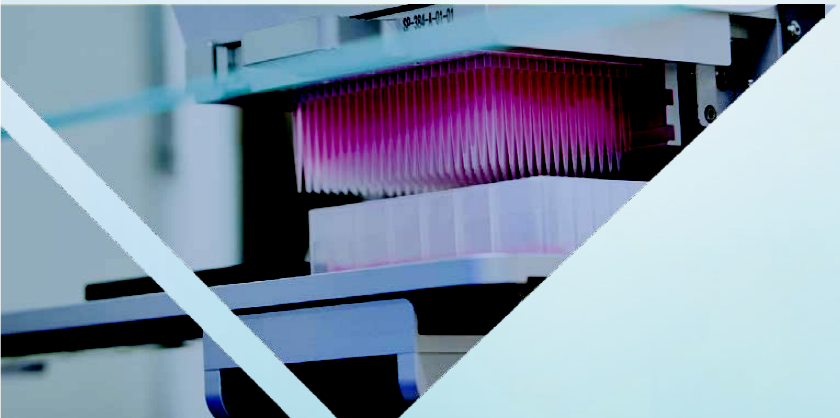
# *in vitro* Low Clearance Studies

Laboratory Services


*Discovery to Development*
*From Insight to Impact*



# Unlock the challenges of low clearance compounds in drug development

## Low Turnover Compound Clearance

Many promising drug candidates are slowly metabolized, leading to low intrinsic clearance ($CL_{int}$) values. Standard short-term hepatocyte assays often fail to capture metabolism of these low turnover compounds, limiting predictions for *in vivo* pharmacokinetics. Inaccurate clearance predictions risk poor dose selection, unexpected accumulation and overlooked safety liabilities. Regulatory agencies increasingly emphasize robust *in vitro - in vivo* extrapolation (IVIVE) strategies to support clinical dose selection and drug-drug interaction (DDI) risk evaluation.

### Our Capabilities:

- Comprehensive low clearance toolkit; multiple assay formats tailored to your needs
- Accurate clearance predictions for challenging drugs
- Industry-leading co-culture models including HμREL® and HEPATOPAC® platforms delivering human-relevant data
- Cross-species capabilities to support for translational studies
- Regulatory-aligned IVIVE strategies to strengthen PBPK modelling and clinical dose predictions
- End-to-end DMPK expertise from discovery to regulatory submission


# Metabolite ID Options

Our state-of-the-art LC-MS/MS platforms and global teams deliver comprehensive profiling of parent compounds and metabolites, even for low clearance drugs where sensitivity and accuracy are critical. From structural elucidation to quantitative analysis, we provide reliable data to support regulatory submissions and accelerate your development pipeline.

## Our Capabilities:

- High-resolution mass spectrometry platforms for sensitive and accurate structural elucidation
- Specialised expertise in challenging matrices and low-abundance metabolites
- Integrated IVIVE studies
- Early discovery metabolite mapping
- Simultaneous QualQuant metabolite identification capabilities for supported low clearance models (plated and suspended hepatocytes, HμREL, and HEPATOPAC)

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# Relay Suspension Hepatocyte and Plated Hepatocyte Models

## Validated Approaches for Low Turnover Compounds

Our relay suspension and plated hepatocyte models provide robust clearance estimates and early metabolite information to improve prediction accuracy for low turnover drugs.

### Relay Human Hepatocytes:

**Incubation Time** = Up to 20 hours, five relays, 6 sample timepoints

**LLoQ** = 0.27 µL/min/million cells

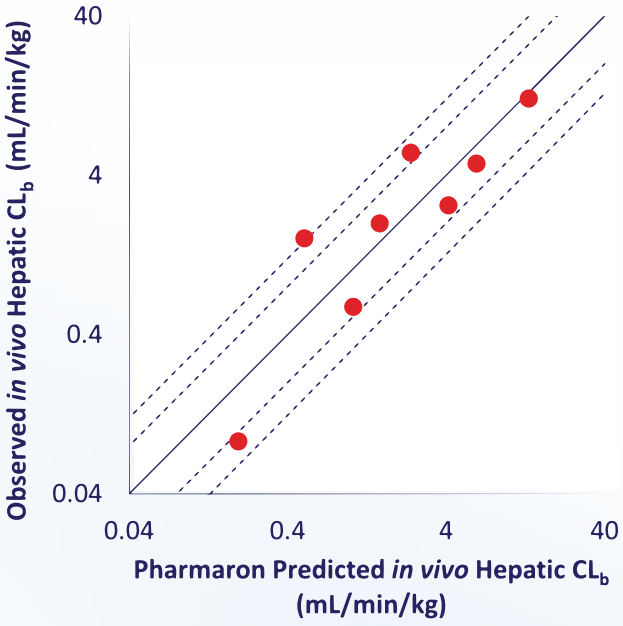
**Format** = 24- and 96-well plate

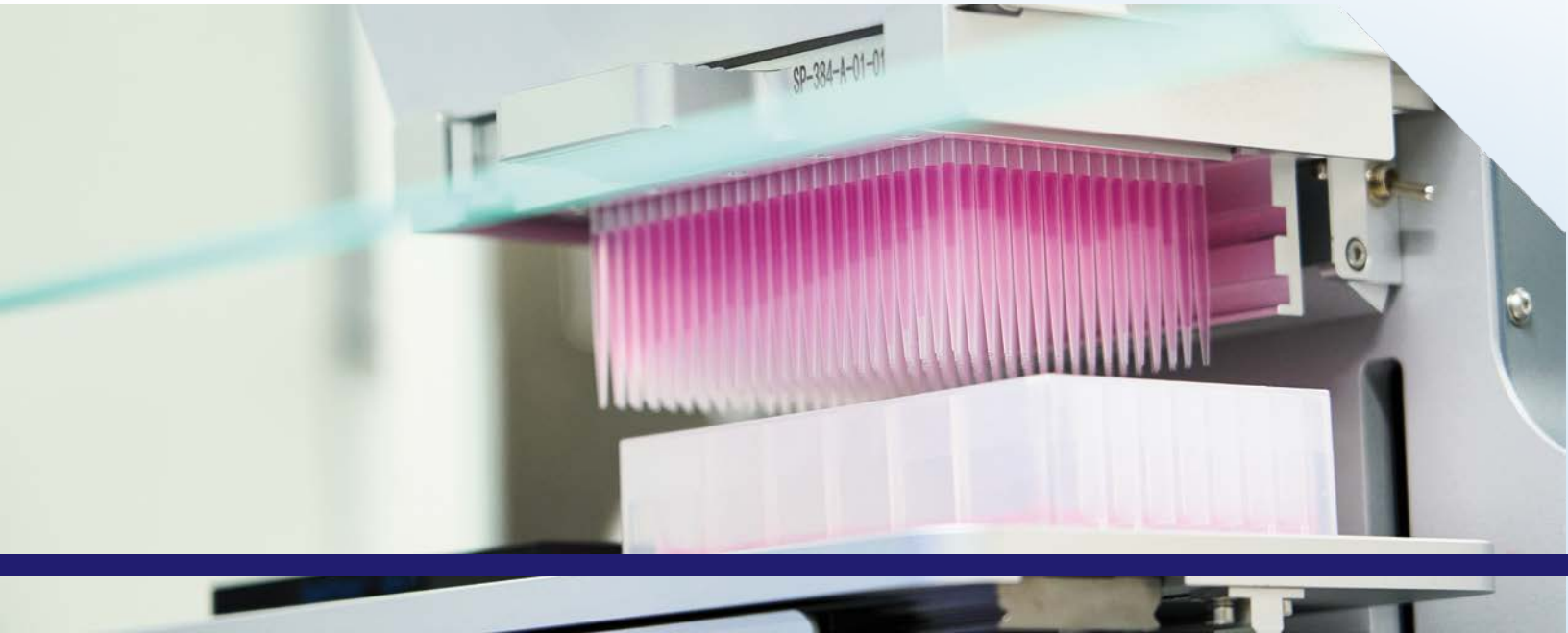
**Validated** = 8 compounds

### Capabilities:

- Cost-effective
- Quantification of metabolic rates
- Metabolite generation and profiling
- Supports human PK predictions through PBPK modeling
- Proven IVIVE reliability
- Validated using multiple industry-standard reference compounds
- Performance qualified within 2–3 fold of observed *in vivo* clearance

### Relay Hepatocytes Validation Set:



## Plated Human Hepatocytes:

**Incubation Time** = 72 hours, 8 sample timepoints

**LLoQ** = 0.1 µL/min/million cells

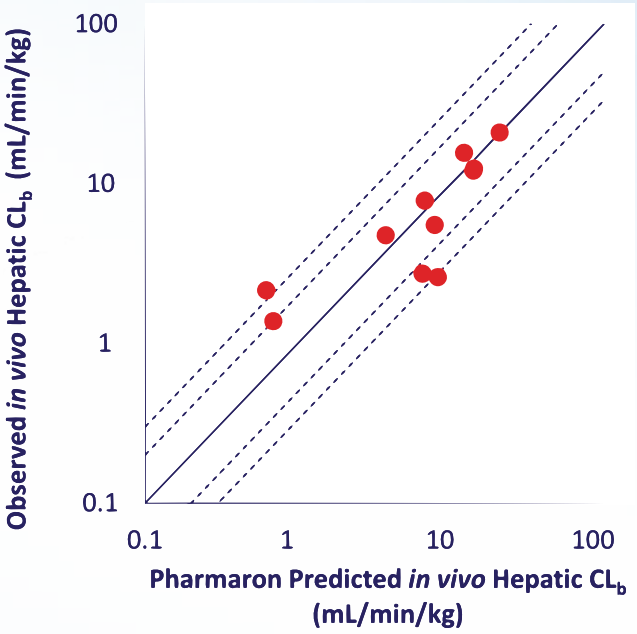
**Format** = 96-well plate

**Validated** = 10 compounds

## Capabilities:

- Multiple species offering: human, monkey, dog, rat, and mouse
- Precise quantification of metabolic rates
- Metabolite generation and profiling
- Supports human PK predictions through PBPK modeling
- Consistent and reliable IVIVE performance
- Validated using multiple industry-standard reference compounds for accuracy

## Plated Hepatocytes Validation Set:




Advanced low clearance systems like the relay suspension and plated hepatocyte models extend metabolic competence and enzyme activity, ensuring accurate characterization of low turnover compounds that would appear stable in conventional assays. They provide improved sensitivity, physiological relevance and reliable measurements which strengthen human PK predication, de-risk dose selection, and support the advancement of low turnover compounds into clinical development.

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# HμREL Micro Livers: Extended Incubation for Predictive Low Clearance Data

HμREL Micro Liver assays combine extended incubation, throughput, and metabolic profiling, enabling accurate predictions for challenging low turnover compounds.

## HμREL Micro Livers:

**Incubation Time** = 120 hours, 4 sample timepoints

**LLoQ** = 0.1 µL/min/million cells

**Format** = 384-well plate

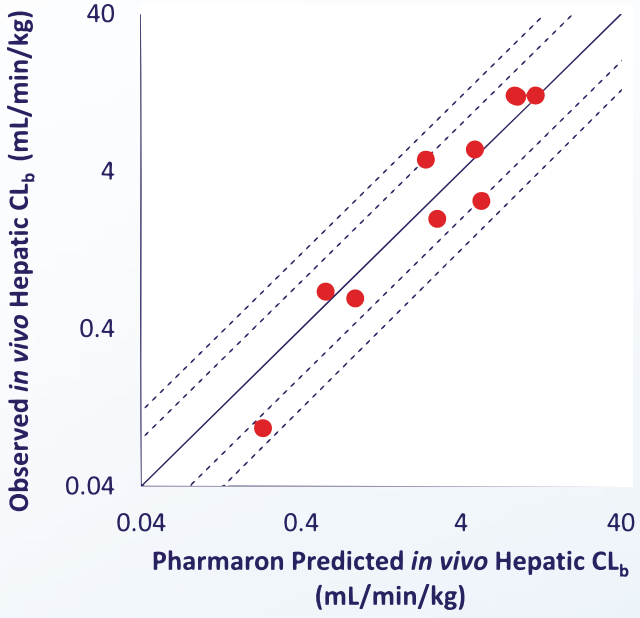
**Validated** = 10 compounds

## Capabilities:

- Clearance quantification across a wide dynamic range, including cytochrome P450 (CYP), UDP-glucuronosyltransferase (UGT), aldehyde oxidase (AO), and flavin-containing monooxygenase substrates (FMO)
- Accurate *in vitro - in vivo* correlation using regression offset calibration
- Integration with Pharmaron's metabolite identification (Met-ID) workflows using LC-MS/MS and advanced QTOF analysis
- Efficient sample use; residual material from clearance assays applied to metabolite identification
- The assay is validated with a group of compounds displaying a range of different clearance values and metabolic pathways

## HμREL Validation Set

Based on linear regression using actual *in vitro* $CL_{int}$ values, the HμREL system was shown to be able to accurately predict *in vivo* data of low clearance compounds.



# HEPATOPAC: Low Clearance Compound Detection

Our HEPATOPAC platform provides a long-term, micropatterned hepatocyte co-culture that maintains metabolic competence for weeks, enabling accurate clearance measurements of low turnover compounds. This industry-validated model delivers human-relevant data to guide dose selection, PBPK modeling and regulatory submissions where conventional assays fall short.

## HEPATOPAC:

**Incubation Time** = 168 hours, 6 sample timepoints

**LLoQ** = 0.1 µL/min/million cells

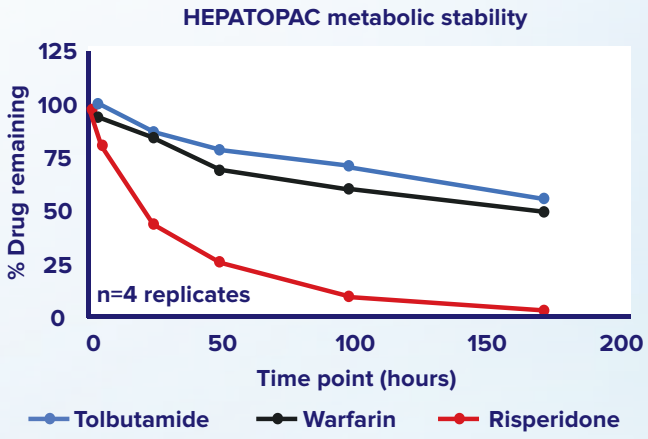
**Format** = 96-well plate

**Validated** = Clearance comparable to published validation[1] using three model compounds

## Capabilities:

- Maintains drug-metabolizing enzyme activity for weeks
- Human-relevant clearance estimates to guide dose selection and IVIVE
- Supported deliverables: *in vitro* half-life, intrinsic clearance, hepatic clearance, metabolite profiling and identification

| Model compound (Clearance classification)) | Observed hepatic clearance from HEPATOPAC (ml/min/kg) | Clearance from literature (ml/min/kg) |
|---|---|---|
| Warfarin (low) | 0.040 | 0.045 |
| Tolbutamide (low) | 0.194 | 0.170 |
| Risperidone (moderate) | 2.03 | 3.56 |




[1] Chan *et al.* Meeting the challenge of predicting hepatic clearance of compounds slowly metabolized by cytochrome P450 using a novel hepatocyte model, HepatoPac. DMD 2019 Jan;47(1): 58_66. doi: 10.1124/dmd.113.053397fullarticlecorrection

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Founded in 2004, Pharmaron is a global life science service provider that offers a broad spectrum of research, development and manufacturing service capabilities throughout the entire drug discovery, preclinical and clinical development process across multiple therapeutic modalities, including small molecules, biologics and CGT products.


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pharmaron.com ■ bd@pharmaron.com