Université de Montréal

Plateforme de biopharmacie

# List of services

2023

Platform of Biopharmacy
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# Physicochemical properties/API characterization

## ❖ In silico predictors

- Physicochemical properties predicted with *in silico* software

## ❖ Particle size by Laser diffraction

- *Instrument :* Coulter LS 13 320
- *Conditions :* 1 sample per compound + 1 standard control, triplicate analysis

## ❖ Particle size by Dynamic light scattering (DLS)

- *Instrument :* Malvern Zetasizer NS
- *Conditions :* 1 sample per compound + 1 standard control, triplicate analysis

## ❖ Particulate matter (in injections, USP 788)

- *Instrument :* Lighthouse LS20

## ❖ pKa determination by acid/base titration

- *Method:* pH-metric titration

## ❖ LogP and LogD determination by Shake-Flask method

- *Method :* Compounds incubated in two-phase system under shaking, samples collected from both phases after equilibration and analyzed by HPLC.
- *Conditions:* 1 sample per compound, triplicate analysis
  pH conditions : pH 1.2, pH 4.5 and pH 6.8.

## ❖ $LogD_{7.4}$ by HPLC-C18 or IAM

- *Method :* Compounds are injected on a column and analysed by HPLC. The retention time on a C18 is proportional to $LogD_{7.4}$. A calibration curve is generated with compounds of known theoretical $logD_{7.4}$ (atenolol, sulipride, metoprolol, labetalol, diltiazem, triphenylen). After a first estimation of logD using the C18, a new analysis can be performed on a lipid IAM column, using an appropriate isocratic elution.

## ❖ Flow properties evaluation

- *Instrument :* Flodex

## ❖ Thermal analysis by DSC and TGA

- *Instruments: Perkin Elmer Jade DSC, TA instrument Q service*

## ❖ Isothermal Titration Calorimetry (ITC)

- *Instruments: ITC GE*

## ❖ Viscosity

- *Instrument:* Brookfield Rheometer

## ❖ Water content

- *Instrument:* Karl Fisher

## ❖ Structural characterization

- XPRD: X-ray powder diffraction.
- FT-IR: Fourier-transfer infrared.
- SEM: Scanning electron microscope
- NMR

### ❖ Chemical stability

- *Method :* test compound is incubated at 1µM (other concentration upon request) with buffer (final DMSO concentration = 0.1%). Remaining compound quantified at various timepoints by LC-MS/MS.
- *Conditions:* Several pHs available (pH 2, pH 6, pH 7.4, pH 10), fasted or fed simulated intestinal fluid (SIF) and simulated gastric fluid (SGF), several temperatures available (4 °C, room temperature or 37 °C)

### ❖ Solubility screening, thermodynamic or kinetic method

- **Thermodynamic (dissolution method)**: involves adding an excess quantity of solid material to an established volume of vehicle. This saturated solution is agitated until equilibrium is reached, generally 24 to 48 hours. Following separation by centrifugation, the compound in solution is analyzed and quantified by HPLC.
  *Conditions: this method allows the evaluation of solubility up to 50 mg/mL concentration using 115 mg of compound for each evaluated vehicle (n = 3); higher concentrations can be evaluated if material is available in larger quantities.*

- **Kinetic (by precipitation)**: the compound is solubilised in DMSO and then injected in different pharmaceutical vehicles. After 4 hours of incubation the mixture is centrifuged. The obtained supernatant is analyzed to determine the exact concentration of the compound.
  *Conditions: this method allows the evaluation of drug solubility up to a concentration of about 1 mg/mL. The amount of powder necessary to carry out the test is about 0.3 mg for each evaluated vehicle (n = 3).*

- **Kinetic (by evaporation)**: the compound is first solubilised in acetonitrile. This solution is dispensed into the wells of a 96-well plate and the acetonitrile evaporated, then 100 µL of vehicle are added to the wells. After 24 hours of incubation the mixture is centrifuged and the supernatant is analyzed by HPLC to determine the exact concentration of the compound.
  *Conditions: this method is limited by the amount of compound available i.e.: 10 vehicles in triplicate correspond to 30 tests. If 50 mg are available, it means 1.6 mg per well. It will allow the evaluation of the solubility up to 16 mg/mL. If tests are performed only once, solubility up to 50 mg/mL could be evaluated.*

## Analytical method development

### ❖ Bioanalytical method

- *Instruments:* LC-MS/MS Qtrap 4000 and Xevo G2-XS QTof
- *Matrices:* Plasma, blood, tissues (brain, heart, lungs, liver, tumors, skin, muscles), CSF, urine, feces, saliva, cells and others
- *Compounds:* Small molecules, challenging molecules, proteins, peptides
- *Extraction:* SPE, LPE, protein precipitation

### ❖ Stability indicating and purity methods

- *Instruments:* HPLC-UV-Fluo-ELSD
- Forced degradation studies

# In vitro metabolism/toxicity

## ❖ Microsomal stability

- *Conditions :* compound (1 µM) incubated in the presence of liver microsomes of various species (0.5 mg/mL) and NADPH for periods of time up to 60 min (7 time points, n=2 per condition). Control incubations are also prepared in the absence of NADPH (60 min, n=1)
- *Species:* human, mouse, rat, dog, monkey
- *Control:* verapamil or loperamide
- *Ouput :* Kinetic profile of drug disappearance (1h), Intrinsic clearance ($Cl_{int}$, µL/min/mg), Half-life

## ❖ Hepatocyte stability

- *Conditions :* compound *(*5 µM) incubated in the presence of cryopreserved liver hepatocytes (1 million cells/mL) for periods of time up to 60 min (5 time points, n=2 per condition). Control incubations are also prepared in KH buffer (60 min, n=1)
- *Species:* human, mouse, rat, dog, monkey
- *Control:* verapamil or loperamide
- *Ouput :* Kinetic profile of drug disappearance (1h), Intrinsic clearance ($Cl_{int}$, µL/min/mg), Half-life

## ❖ S9 stability

- *Conditions :* compound *(*1 µM) incubated in the presence of liver S9 of various species (1 mg/mL) and NADPH, UDPGA or PAPS for periods of time up to 60 min (7 time points, n=2 per condition). Control incubations are also prepared in the absence of cofactor (60 min, n=1)
- *Species :* human, mouse, rat, dog, monkey
- *Control :* midazolam (phase 1) and 7-hydroxycoumarin (phase 2)
- *Ouput :* Kinetic profile of drug disappearance (1h), Intrinsic clearance ($Cl_{int}$, µL/min/mg), Half-life

## ❖ Metabolite profiling

- In microsomal or hepatocyte incubations (other matrices on demand)
- Identification of metabolites by LC-MS/MS (Sciex QTrap 4000) and MS/MS fragmentation

## ❖ Metabolite ID

- In vitro and in vivo
- Identification of metabolites with HRMS Xevo G2-XS (QTof)

## ❖ Targeted metabolomics

- Identification, quantification and comparison of metabolites by UPLC-QTof

## ❖ Reactive metabolite trapping (GSH)

- Microsomal incubation in presence and absence of NADPH and GSH
- Detection of the conjugates by LC-MS/MS or QTof

## ❖ Plasma stability

- *Conditions :* compound (1 µM) incubated in the presence of plasma from various species for periods of time up to 120 min (5 time points, n=2 per condition).
- *Species :* human, mouse, rat
- *Control :* procaine (human), enalapril (rat)
- *Ouput :* Kinetic profile of drug disappearance (2h), Half-life

## ❖ Plasma protein binding

- *Conditions :* compound (1 µM, other upon request) incubated in plasma (100, 50 or 10%) dialysed against buffer in HTDialysis system, for 5h at 37°C, 5% $CO_2$, (n=3 per condition). Control incubations are also prepared in buffer against buffer at 10% of the tested concentration in plasma, to test for solubility or non-specific binding issues.
- *Alternative :* for compounds unstable in plasma, the incubation can be performed in human serum albumin and /or α1-acidglycoprotein
- *Species :* human, mouse, rat, dog, monkey
- *Control :* propranolol
- *Ouput :* Free fraction

## ❖ Microsomal protein binding

- *Conditions :* compound (1 µM, other upon request) incubated in liver microsomes (0.5 mg/mL, or as requested) dialysed against buffer in HTDialysis system, for 5h at 37°C, 5% $CO_2$, n=3 per condition). Control incubations are also prepared in buffer against buffer at 10% of the tested concentration in plasma, to test for solubility or non-specific binding issues.
- *Species :* human, mouse, rat, dog, monkey
- *Control :* propranolol
- *Ouput :* Free fraction

## ❖ Tissue homogenate protein binding

- *Conditions :* compound (1 µM, other upon request) incubated in tissue homogenate, dialysed against buffer in HTDialysis system, for 5h at 37°C, 5% $CO_2$, (n=3 per condition). Control incubations are also prepared in buffer against buffer at 10% of the tested concentration in plasma, to test for solubility or non-specific binding issues.
- *Species :* mouse, rat
- *Control :* midazolam
- *Ouput :* Free fraction in tissue (corrected with dilution factor)

## ❖ P450 competitive inhibition (reversible)

- *Conditions :* 4 isoforms tested (CYP3A4, CYP2D6, CYP1A2, CYP2C9). Compound incubated at 9 different concentrations (2.2nM – 15 µM), in human liver microsomes (0.25 – 0.5mg/mL depending on isoform), with NADPH and specific substrate.
- *Controls:* isoform specific (see table below)
- *Ouput :* $IC_{50}$

*Table of CYPinhibition assay conditions*

| | CYP2D6 | CYP1A2 | CYP2C9 | CYP3A4 | CYP2C19 |
|---|---|---|---|---|---|
| Substrates | Dextromethorphan 15µM | Phenacetin 100µM | Diclofenac 10µM | Testosterone 50µM | S-Mephenytoin 10µM |
| Control inhibitors | Quinidine 0.15nM à 1.5µM | α-naphthoflavone 0.15nM à 1.5µM | Miconazole 1.5nM à 15µM | Ketoconazole 0.8nM à 5µM | S-Benzylnirvanol 1.5nM to 15µM |
| Metabolites | Dextrorphan | Acetaminophene | 4'-OH-diclofenac | 6β-OH-testosterone | 4'-OH-mephenytoin |
| Conc. HLM | 0.25 mg/mL | 0.50 mg/mL | 0.25 mg/mL | 0.25 mg/mL | 0.25 mg/mL |
| Incubation time | 20 min | 20 min | 10 min | 10 min | 10 min |
| Internal standard | Labetalol | Metoprolol | Carbamazepine | Loratadine | labetalol |

## ❖ P450 time-dependent inhibition (irreversible)

- *Conditions :* 1 isoform tested (CYP3A4). Compound incubated at 9 different concentrations (2.2nM – 15 µM), in human liver microsomes (0.25 – 0.5mg/mL depending on isoform), with specific substrate, after 30min pre-incubation +/- NADPH.
- *Controls:* mifepristone
- *Ouput :* $IC_{50}$ shift

# In vitro permeability

## ❖ Caco-2 permeability

- *Conditions*: Caco-2 cells are grown to confluence on porous filter support of the cell culture insert (24-well plate) over approximately 21 days.Test compound is placed on the apical side of the cell layer and diffuses into the basolateral compartment (pH gradient). To study drug efflux, the transport from basolateral to apical compartment is also investigated. Aliquots from the receptor compartments are removed at specific time points (0, 1, 2 and 3 hours). Each compound is evaluated in triplicate, and the concentration is determined by LC-MS/MS. Upon request, verapamil can be co-incubated with the test compound to identify if P-gp transporters are responsible for the drug efflux.
- *Controls*: atenolol (paracellular transport, low permeability), metoprolol (passive transcellular transport), digoxin (pgp substrate, to confirm pgp expression)
- *Output*: Kinetic profiles of basolateral/apical concentrations for each test compound, Permeability coefficient $P_{app}$, Efflux ratio i.e. $P_{app}$(B-A)/$P_{app}$(A-B)

## ❖ MDCK-MDR1 P-glycoprotein inhibition

- *Conditions*: MDCK-II MDR1 cells are grown to confluence on porous filter support of the cell culture insert (24-well plate) over 4 days. Transport of substrate (loperamide or quinidine) apical to basolateral and basolateral to apical is evaluated over 60 minutes in the presence or absence of test compound at various concentrations (0 to 100 µM). Substrate concentration in each compartment is determined by LC-MS/MS. Efflux ratios are calculated and the regression of efflux ratios over test compound concentration is used to calculate an IC50.
- *Controls*: ketoconazole (P-glycoprotein inhibitor), MDCK-II wild type cells (negative control)
- *Output*: Permeability coefficient $P_{app}$ A-B and B-A, Efflux ratio i.e. $P_{app}$(B-A)/$P_{app}$(A-B), IC50

## ❖ PAMPA (Kansy method)

- *Conditions*: based on diffusion across an artificial lipidic membrane, obtained by soaking an hydrophobic filter in solution of phospholipids dissolved in n-dodecane during 5 min. Phosphate buffer containing test compound and controls is transferred in donor side, and phosphate buffer alone is placed in receiver side. Incubation takes place during 16 h, at 37 °C, 50 rpm.
- *Controls*: cassette of 8 compounds covering all BCS classes
- *Output*: Kinetic profiles of diffusion, Permeability coefficient $P_{app}$

## ❖ PAMPA-BBB (Kansy method)

- *Conditions*: similar as above, but replacing the phospholipids by brain polar lipid extract (porcine).
- *Controls*: cassette of 7 compounds covering high and low BBB permeation ranges (CNS +/-)
- *Output*: Kinetic profiles of diffusion, Permeability coefficient $P_{app}$

## ❖ Diffusion *in vitro* (Franz cells)

- *Conditions*: compound in donor side, 10mM PBS buffer pH 7.4 in receiver side. Controlled temperature (22 to 37 °C), 300 rpm, 18 mM diameter membranes (polycarbonate, cellulose, nylon, other upon request), triplicate analysis, horizontal or vertical diffusion. Sampling at various timepoints, quantification by HPLC.
- *Control*: ibuprofen
- *Output*: Kinetic profiles of diffusion, diffusion constant.

# *In vivo* PK studies

- *In vivo* part performed at the Animalerie Jean et Marcel Coutu (AJMC) at IRIC. Bioanalysis performed at the platform.
- Species available : mouse and rat (any strain)
- Dosing : PO, IV and other available (IP, IM, etc.)
- Typical dose : 1 mg/kg iv, 10 mg/kg po
- Tissue collection possible (liver, heart, muscle, brain, other on demand)
- Metabolic cages available (urine and feces collection)
- Data analysis : Kinetica Software for PK/PD Data Analysis, Simulation and Reporting (Thermo Fisher)

# Formulation/nanoformulation and stability

Preformulation development adapted to your needs

- Tablets, capsules, liquids, enterocoated tablets, etc.
- Preparation of nanoformulations :
  - liposomes or nanoparticles, by extrusion or emulsification
  - nanocristals obtained by nanomilling (high or low energy)
  - polymeric nanoparticles obtained by high pressure homogenisation or nanoprecipitation
  - nanoemulsions
- Nanoparticles functionalization
  - Surface modification of nanoparticles (biocompatible polymers)
  - Grafting of specific targeting elements (ligands, antibodies, aptamers) pour cellular penetration
  - Fluorescent markers for intracellular or biodistribution tracking
- Stability studies ICH conditions (25°C/60%HR, 30°C/65%HR, 40°C/75%HR)
- Content uniformity, forced degradation, dissolution etc…

# Assays to be validated on demand*

- **CYP inhibition (competitive) : other isoforms (CYP2B6, CYP2C8, CYP2C9)**
- **CYP inhibition (irreversible) : other isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6)**
- **CYP induction using CYP450 Protein Assay – Human Induction Kit (1A2, 2B6, 3A4, and 3A5)**
- **CYP and UGT reaction phenotyping**
- **Blood/plasma ratio**
- **In vitro hemolysis assay (spectrophotometric)**
- **AMES test (mutagenic potential)**
- **Cytotoxicity (MTT)**

*Assays used to be performed by our experts in previous industry settings, not yet validated at the platform.

## Our team :

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## How to find us ?

The Biopharmacy platform is located in the Jean Coutu building of Université de Montréal.

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By car : parking available in the Louis-Colin garage, indicated in blue (free under 30min)