Methodology for In Vitro to In Vivo Extrapolation (IVIVE)
Notation for R code: "`IVIVE_R_Code_v1_2020916.R`"

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Objective

The objective of this methodology is to predict the steady-state concentration of a chemical substance in a target tissue (e.g., liver) in vivo, using a partition coefficient derived from a simple in vitro experiment with relevant cells (e.g., hepatocytes). The predicted tissue concentration is then compared to an observed in vivo concentration to validate the model and understand the underlying distribution mechanisms of the substance.

Part 1: The In Vitro Experiment and Partition Coefficient Derivation

An experiment is conducted using cryopreserved cells, such as human hepatocytes, to determine the partitioning behavior of the substance between the experimental medium and the cells.

Step 1.1: Calculate In Vitro Concentrations

First, the necessary volumes and concentrations from the experimental setup are calculated.

- Total Cell Volume ($V_{cell,vitro}$): The total volume of all cells in the experiment is calculated by multiplying the number of cells by the mean volume of a single cell.

$$V_{cell,vitro} = \text{Number of Hepatocytes} \times V_{cell,single}$$

- Free Substance Concentration in Medium ($C_{free,medium}$): The concentration of the substance that is not bound to protein in the medium is calculated by dividing the measured mass of the free substance by the volume of the medium.

$$C_{free,medium} = M_{free,medium}/V_{medium}$$

- Total Substance Concentration in Cells ($C_{cell,vitro}$): The total concentration of the substance inside the cells is calculated by dividing the measured mass of the substance in the cells by the total cell volume.

$$C_{cell,vitro} = M_{cell,vitro}/V_{cell,vitro}$$

Step 1.2: Calculate the In Vitro Partition Coefficient ($PC_{invitro}$)

The key parameter derived from the in vitro experiment is the partition coefficient. This value represents the tendency of the substance to accumulate in the cells relative to the free concentration in the surrounding fluid at equilibrium.

- Equation: The partition coefficient is calculated as the ratio of the total concentration in the cells to the free concentration in the medium.

$$PC_{invitro} = C_{cell,vitro}/C_{free,medium}$$

This unitless partition coefficient is the critical value that will be extrapolated for use in the in silico model.

Part 2: The In Silico Prediction of In Vivo Concentration

The $PC_{invitro}$ value is now used within a mathematical model to predict the substance's concentration in a target tissue in vivo, starting from the observed concentration in the blood.

Step 2.1: Convert Units and Calculate Free Concentration in Blood

The observed total concentration of the substance in blood, typically measured in mass units (e.g., μg/L), is first converted to molar concentration (e.g., nmol/L) using the substance's molar mass.

- Equation: The free concentration of the substance in the blood is then calculated. This equation accounts for the binding of the substance to proteins (e.g., albumin) in the blood plasma.

$$C_{free,blood} = C_{blood,obs}/(1 + K_a \times C_{protein,blood})$$

Step 2.2: Apply IVIVE Hypothesis and Calculate Cellular Concentration

The core assumption of this IVIVE model is that the free concentration of the substance in the blood is in equilibrium with the free concentration in the tissue's interstitial fluid (ISF).

- IVIVE Hypothesis:

$$C_{free,ISF} = C_{free,blood}$$

- Equation: This free ISF concentration is then used, along with the partition coefficient derived in vitro, to predict the total concentration of the substance inside the tissue's cells in vivo.

$$C_{cell} = C_{free,ISF} \times PC_{invitro}$$

Step 2.3: Calculate Total Predicted Tissue Concentration ($C_{tissue,pred}$)

The final step is to perform a mass balance calculation to determine the total predicted concentration in the tissue. This involves summing the mass of the substance in each of the tissue's sub-compartments (residual blood, interstitial fluid, and cells) and dividing by the total tissue volume.

- First, calculate the Total ISF Concentration ($C_{total,ISF}$): This is required to find the total mass in the ISF, accounting for protein binding.

$$C_{total,ISF} = C_{free,ISF} \times (1 + K_a \times C_{protein,ISF})$$

- Next, calculate the mass in each sub-compartment:

$$M_{blood} = C_{blood,obs} \times V_{blood}$$
$$M_{ISF} = C_{total,ISF} \times V_{ISF}$$
$$M_{cell} = C_{cell} \times V_{cell}$$

- Finally, calculate the Predicted Tissue Concentration ($C_{tissue,pred}$): The total mass is the sum of the sub-compartment masses, which is then divided by the total tissue volume.

$$C_{tissue,pred} = (M_{blood} + M_{ISF} + M_{cell})/V_{tissue}$$

The final predicted concentration, typically in molar units, is converted back to mass units (e.g., μg/kg) using the molar mass for direct comparison with the observed value.

Part 3: Comparison, Analysis, and Conclusion

The predicted tissue concentration ($C_{tissue,pred}$) is compared against the experimentally observed tissue concentration ($C_{tissue,obs}$).

- If $C_{tissue,pred} \approx C_{tissue,obs}$: The model is considered validated. This suggests that the substance's distribution is primarily governed by passive partitioning and protein binding, and that active transport mechanisms are not significant.

- If $C_{tissue,pred} > C_{tissue,obs}$: The model has over-predicted accumulation. This suggests the presence of active efflux transporters in the tissue that pump the substance out of the cells, limiting its net accumulation in vivo.

- If $C_{tissue,pred} < C_{tissue,obs}$: The model has under-predicted accumulation. This suggests the presence of active uptake transporters that pull the substance into the cells, leading to higher concentrations in vivo than predicted by passive processes alone.

Notation

Parameters (Known or Measured Values)

Symbol	Description	R Code Symbol
Hepatocytes	Number of cells used in the in vitro experiment	n_hepatocytes
$V_{cell,single}$	The mean volume of a single hepatocyte	vol_cell_single_um3
V_{medium}	The volume of the medium in the in vitro experiment	vol_medium_mL
$M_{cell,vitro}$	Measured mass of the substance in the cells (in vitro)	mass_cell_vitro_nmol
$M_{free,medium}$	Measured mass of the free substance in the medium (in vitro)	mass_free_medium_nmol
$C_{blood,obs}$	Observed total concentration of the substance in blood (in vivo)	c_blood_obs_ug_L
$C_{tissue,obs}$	Observed total concentration of the substance in tissue (in vivo)	c_tissue_obs_ug_kg
MM	Molar Mass of the substance	mm_compoundX_g_mol
K_a	Affinity constant of the substance for albumin	Ka_compoundX_albumin_L_mol
$C_{protein,blood}$	Total concentration of protein (albumin) in blood plasma	c_protein_blood_uM
$C_{protein,ISF}$	Total concentration of protein (albumin) in interstitial fluid	c_protein_ISF_uM
V_{blood}	Volume fraction of residual blood in the target tissue	vol_fraction_blood
V_{ISF}	Volume fraction of interstitial fluid in the target tissue	vol_fraction_ISF
V_{cell}	Volume fraction of cells in the target tissue	vol_fraction_cell
V_{tissue}	Total volume of the representative tissue sample	vol_tissue_L

Variables (Calculated Values)

Symbol	Description	R Code Symbol
$V_{cell,vitro}$	Total volume of cells in the in vitro experiment	vol_cell_vitro_L
$C_{free,medium}$	Free concentration of the substance in the medium (in vitro)	c_free_medium_nM
$C_{cell,vitro}$	Total concentration of the substance in the cells (in vitro)	c_cell_vitro_nM
$PC_{invitro}$	The calculated in vitro partition coefficient	PC_in_vitro
$C_{free,blood}$	Free concentration of the substance in blood (in vivo)	c_free_blood_nM
$C_{free,ISF}$	Free concentration of the substance in interstitial fluid (in vivo)	c_free_ISF_nM
C_{cell}	Predicted total concentration of the substance in tissue cells (in vivo)	c_cell_nM
$C_{total,ISF}$	Total concentration of the substance in interstitial fluid (in vivo)	c_total_ISF_nM
M_{blood}	Mass of the substance in the residual blood of the tissue sample	mass_blood_nmol
M_{ISF}	Mass of the substance in the interstitial fluid of the tissue sample	mass_ISF_nmol
M_{cell}	Mass of the substance in the cells of the tissue sample	mass_cell_nmol
$C_{tissue,pred}$	Final predicted total concentration of the substance in the tissue	c_tissue_pred_ug_kg