# User Manual: Procedure for Applying Framework

## Tool Reference

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# Procedure for Applying Framework

The instructions below describe the models used for estimating liquid-liquid extraction recovery. The instructions also include a procedure for how to apply the models to establish the impact of liquid-liquid extraction method parameters on recovery of extractables in a chemical characterization study.

## 1. Define the Chemical Space and Chemical Subspace

The **chemical space** refers to a comprehensive list of all possible extractables which can be found in the chemical characterization study. The **chemical subspace** refers to a subset of the chemical space applicable to the specific analysis within the chemical characterization study. For example, for liquid-liquid extraction as part of gas-chromatographic analysis of semi-volatile extractable chemicals, a chemical subspace may pertain to only chemicals within the chemical space with a boiling point of 50°-450°C.

The user must determine their own appliable chemical space and chemical subspace based on the intent of the analysis. In establishing the chemical space and chemical subspace, consideration must be made to ensure a comprehensive coverage of chemicals which are applicable to needs of the specific chemical characterization study. The chemical subspace described in the supporting publication, is a general chemical subspace applicable to liquid-liquid extraction when used for gas chromatographic analysis of medical device extractables, and can be initially used.

## 2. Select an effective recovery model

For each chemical within the chemical subspace, recovery for liquid-liquid extraction should be determined using the following equation:

$$Recovery\ (\%) = \left(1 - \left[\frac{1}{1 + K_D(V_E/V_O)}\right]^n\right) \text{x}100$$

Where:

$K_D$ = the distribution coefficient of a given solute

$V_E$ = The volume of aqueous extract

$V_O$ = the volume of organic solvent (dichloromethane, hexanes, or ethyl acetate)

n = the number of exchange iterations used in the liquid-liquid extraction procedure

The values of $V_E$, $V_O$, and n are determined from the experimental conditions selected for performing the liquid-liquid extraction.

Determination of $K_D$ can be accomplished through the use of an appropriate predictive model:

$$\log K_D = \log P - \log\left(1 + 10^{(pH-pKa)\delta_i}\right)$$

Where:

logP = the partition coefficient of the chemical in the subspace

pH = the pH of the aqueous extract (determine from the experimental conditions)

pKa = the pKa of the chemical in the subspace

$\delta_i$ = 1 or -1 for an acidic solute or basic solute, respectively.

The pKa and logP can be experimentally determined or predicted using quantitative structure-propery relationship models. As described in the publication, validated prediction models have been previously established for estimation of these parameters and which are available in the public domain.

**3. Select surrogate chemicals to bracket relevant physiochemical parameters**

From the chemical subspace, determine the recoveries of each solute using the recovery model established in step 2 based on the experimental conditions being used. Choose at least three surrogate compounds (one acidic, one basic, one neutral) which is expected to show a recovery of <10%, three with expected recovery between 40-60%, and three surrogate with expected recovery of >90%. A minimum of nine surrogates should be used.

**4. Demonstrate predicted recoveries empirically**

Perform liquid-liquid extraction on the surrogate compounds selected in step 3 using the predetermined experimental parameters. Verify the recoveries obtained for low (<10%) and high (>90%) recovery are within 20% of the expected values based on the model. The middle recovery (40-60%) should align with the inflection of the recovery curve, as showin in Fig. 1.

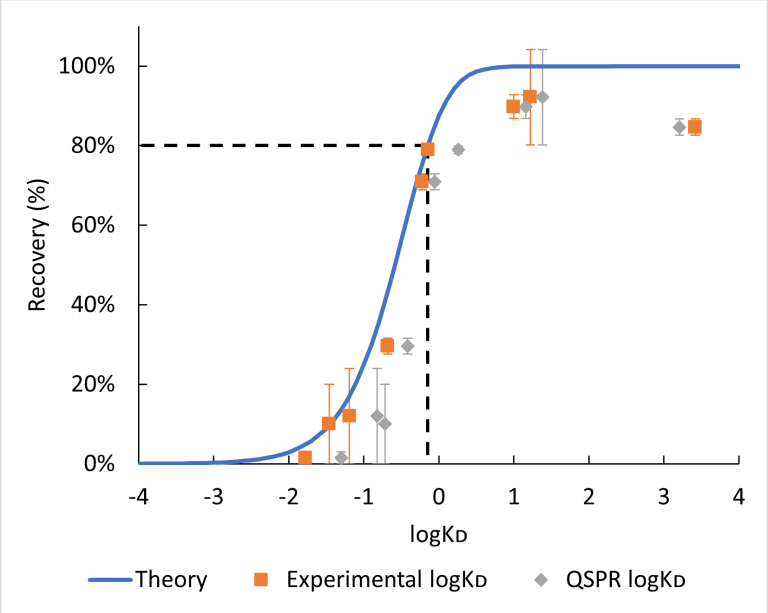


*Fig. 1 Example recovery plot for a liquid-liquid extraction method. The orange squares are experimentally determined log$K_D$ values. The gray diamonds are QSPR determined log$K_D$ values. The black dashed line is the recovery cutoff (less than 80% recovery is considered unrecovered).*

If the recoveries obtained are within the expected range, the liquid-liquid extraction method and model utilized is functioning as expected. The recoveries calculated for the chemical subspace in step 3 can be used to estimate the impact of the method on recovery of the intended chemical space. Establish an 80% acceptable recovery limit (as shown in Fig.1) and determine the number of chemicals within the chemical subspace achieve this minimum value. Adjust the experimental parameters as necessary to maximize coverage of the chemical space. If the experimental parameters are adjusted to values outside of what was evaluated in step 4, step 4 should be repeated to demonstrate method performance using the new values.

If recoveries obtained are not within the expected range, the impact of the chosen liquid-liquid extraction parameters cannot be estimated. Attempts should be made to refine the model to align with laboratory results or refine laboratory technique to align with the selected model. If either is chosen, the adjustments must be verified by repeating steps 3 and 4.

**Reference:**

1. Duelge, Kaleb & Young, Joshua. (2023). Estimating Recovery in the Liquid–Liquid Extraction Chemical Space. Biomedical Materials & Devices. 2. 10.1007/s44174-023-00123-7. https://doi.org/10.1007/s44174-023-00123-7