Shake-Flask Aqueous Solubility Assay (kinetic or thermodynamic assay conditions)

Background: Determining compound solubility is an essential tool for early stages of the drug discovery process, as well as for lead optimization. Low solubility can lead to unpredictable and unreliable results during in vitro testing, thereby increasing the development costs. Solubility issues at the later stages of the drug discovery may lead to poor bioavailability, underestimated toxicity and other obstacles, lowering the chances of a given drug candidate for success. Solubility can be measured either as a kinetic or thermodynamic value. Typically, for early-stage drug discovery the kinetic solubility method is used, as it is fast and well suited for HTS format. In this case, solid compounds are first dissolved in DMSO and then linear serial dilutions of each compound are added to an aqueous buffer and observed for precipitate formation when the compound is not completely soluble. Precipitate appearance can be evaluated by light scattering (laser nephelometry method). For better precision, the solution can be subjected to high-speed centrifugation or filtration using special solubility filter plates and then the compound concentration is measured in the saturated solution directly by UV or LC-MS using separately built calibration curves. Thermodynamic solubility is important for lead optimization and drug formulation stages. It is usually determined for pure compounds: crystalline powders, amorphous substances and liquids. In this modification of solubility assay long (24 hours or more) incubations are required. Measurements are usually performed by the shakeflask method with UV-Vis or LC-MS detection.

Service Details: This commonly used shake flask protocol is based on the use of Millipore Multiscreen solubility filter plates or centrifugation followed by UV-Vis quantitation of dissolved compounds. Microplate reader SpectraMax Plus (Molecular Devices) is used in our lab for UV-Vis measurements. LC-MS/MS quantitation (API3000 mass-detector, AB Sciex) can also be done for poorly UV-absorbing compounds, mixtures, and compounds prone to degradation. Kinetic solubility measurements are performed starting from DMSO stock solutions of the test articles; powders are used for thermodynamic solubility measurements.

Typical assay conditions are as follows:

- Kinetic solubility measurements: 2 h shaking time at 25°C in an aqueous buffer;
- Thermodynamic solubility measurements: 4 h and 24 h shaking time at 25°C.

The assay is run in duplicates. One or two reference compounds are included in each test batch. Assay/ protocol customization is available upon request.

Deliverable: Full study report is provided, including solubilities and calibration curves for test and reference compounds.

Sample Submission: Sample requirement is at least 50 µL of 20 mM stock compound solutions in DMSO for kinetic solubility measurements, and 2 x 4 µmoles of dry compound for thermodynamic solubility measurements. We do not need to know structures of the molecules for ADME testing. However, brutto formulas have to be provided for all studies involving MS detection.